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JOURNAL OF DAIRY SCIENCE

VOLUME XIX OCTOBER, 1936 NUMBER 10

THE PHOSPHOLIPIDS IN MILK

IV. THEIR CHEMICAL NATURE AND THEIR DISTRIBUTION AMONG SOME MILK PRODUCTS

GEO. E. HOLM, P. A. WRIGHT, AND E. F. DEYSHER

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The phospholipid content of milk and various dairy products has been determined by a number of investigators (1). The great variations in the values recorded can no doubt be accounted for on the basis of different methods used in the isolation of the lipid material.

The present authors in their former study of milk (1) used the method of Bloor, as modified by Meigs (2) and used by him in the study of blood lipids, for the separation of this material. This method consists of an extraction with a mixture of 3 parts of 95% ethanol and 1 part of ether. Though the method seems to give excellent results when used in blood studies, it does not seem adaptable to milk or milk products without some modification because of the slight solubility of phosphates in the extraction mixture. Hence in the work on milk referred to, the values given for the percentage phospholipid content of milk as well as of its products were too great, except for those cases wherein the determinations were carried out on the pure lipid material isolated in the various fat tests studied.

This was revealed by our subsequent work, the results of which led to the conclusion (3) "that the values for the phospholipid content of milk of the order of those obtained by Mohr, Brockmann and Müller represent the phospholipid content of milk most accurately."

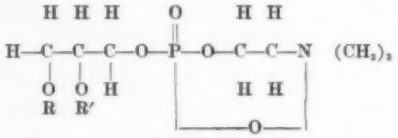
Wiese, Nair and Fleming (4) and Perlman have used the Mojonnier modification of the Roese-Gottlieb method for the extraction of lipid material from cream. The former report 18–20 mgs. of phosphorus in 100 grams of extract while the latter reports values of 14.5 and 15 mgs. in similar extracts from creams of approximately 40% fat content. Horrall (6) using a similar extraction method has determined the phospholipid content of milk and some of its products. The values obtained are generally in agreement in their order of magnitude with those of Mohr, Brockmann and Müller (13).

Received for publication May 1, 1936.

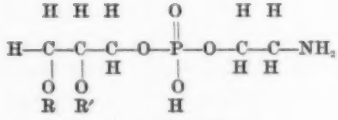
CHEMICAL NATURE OF PHOSPHOLIPID MATERIAL IN MILK

The percentage of phospholipids is usually calculated from the phosphorus content of the material analyzed. Though it has been shown by Osborne and Wakeman (8), Osborne (9) and Koch and Woods (7) that the phospholipids of milk are composed of a mixture of phospholipids the factor used for the conversion is usually calculated upon the assumption that the material is a distearyl or an oleo-stearyl lecithin.

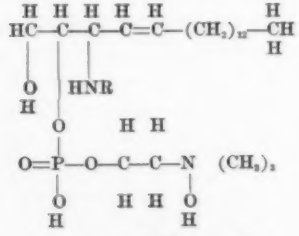
In a study of the material isolated from fresh dry buttermilk Kurtz, Jamieson and Holm (10) found that it was composed of lecithin, cephalin, sphingomyelin present in the ratio of 8.4 : 4.5 : 1, respectively. The general structural formulas of these compounds are as follows.



Lecithins



Cephalins



Sphingomyelins

Cerebrosides were also found present but since these contain no phosphorus they are of no concern here.

The material was further purified and then separated into a fraction containing lecithin and cephalin and one composed of sphingomyelin and cerebrosides. These have been analyzed for their fatty acid constituents in order to determine as accurately as possible the phosphorus ratio in the composite material.

Analysis of the lecithin-cephlin fraction indicated that its constituency of fatty acids was of the following nature and proportion (10).

Myristic	5.2%
Stearic	16.1
Archidic	1.8
Oleic	70.6
Dicostetrenoic (?)	6.3

The acids of the cephalin-sphingomyelin fraction are composed almost entirely of lignoceric acid. From analyses it was calculated that 60.43% of the total isolated material was lecithin, 32.37% was cephalin and 7.20% was sphingomyelin. The average molecular weight of the lecithin-cephalin fraction was calculated to be 773.6, on the basis of 8.4 parts of lecithin to each 4.5 parts of cephalin.

On the basis of unpublished data on the analysis of the acid content of the sphingomyelin-cerebroside fraction the molecular weight of the sphingomyelin was calculated as 805. The distribution of lecithin-cephalin to sphingomyelin was 12.9 to 1.0. On this basis the average molecular weight of the phospholipid material would be 775.76, or slightly higher than would be the case if the phospholipid were a lecithin of the oleo-stearyl or distearyl type. Phosphorus represents 4% of this weight and the factor for conversion of phosphorus to milk phospholipids would therefore be 25.00. This factor has therefore been used tentatively in conversion of the organic phosphorus in the various samples into percentage of phospholipids.

EXPERIMENTAL

Extraction of lipid material: The isolation of phospholipid material from milk or its products has been accomplished by various solvents. The method of Bloor utilizes a mixture of ether and ethanol. Broderick-Pittard's method employs an ether-ethanol mixture for the first extraction with a subsequent extraction with chloroform, after desiccation. Wiese, Nair and Fleming, and Horrall extracted the lipid material by the Mojonnier and Troy modification of the Roese-Gottieb method.

The choice of solvent to be used in this work was determined through a consideration of the properties of the material isolated by Kurtz, Jameson and Holm (10). This material was purified and separated into an ether-soluble and ether-insoluble fraction as described. One consisted of lecithin and cephalin while the other was evidently a mixture of sphingomyelin and cerebrosides.

The approximate solubilities of these two have been determined as given in Table 1.

In view of these results the most thorough extraction of phospholipid material should be accomplished with a combination of solvents; perhaps in the manner in which they were employed by Broderick-Pittard (11).

The results indicate also that cold ethanol-ether or cold ethanol-

TABLE 1
Solubilities of phospholipid fractions in various organic solvents

	ETHER	PET. ETHER	ETHANOL (95%)	$CHCl_3$
Lecithin-cephalin	v. sol.	v. sol.	Somewhat sol. (room temp.)	v. sol.
Sphingomyelin-cerebroside	prac. insol.	insol.	1.90 (room temp.) 13.0 (hot)	20.0 (room temp.) v. sol. (hot)

petroleum ether mixtures are not extremely efficient solvents for the sphingomyelin-cerebroside fraction.

In view of these data the extractions were carried out, as follows:

Whole milk, skim milk, buttermilk, cream: 100 cc. of product were run dropwise from a graduated pipette into a constantly agitated (by rotation) mixture of 375 cc. absolute ethanol and 125 cc. USP ether at room temperature. The contents of the receiving flask were heated to active boiling on the steam bath and filtered, the filtrate being secured in a 16 oz. glass mortar. The precipitate was removed from the flask with small portions of ethanol, ether and chloroform. After thorough drainage the precipitate was removed from the filter paper with a spatula and mixed thoroughly with Na_2SO_4 (anhydrous) and a small amount of white sea sand.

The mortar containing the filtrate and washings was placed in a desiccator, without the porcelain plate, and the water-ether-ethanol-chloroform mixture evaporated by impinging up the surface of the liquid a gentle stream of air, filtered through absorbent cotton and heated by passing through a metal receptacle placed upon a hot plate, with the current switch turned to the "high" position. Usually two seven-hour periods were necessary to complete the evaporation, as the last traces of water evaporated with difficulty. With the exception of the cream which required special treatment before the chloroform extraction, the dry precipitate–sand–Na_2SO_4–mixture, was transferred to this latter mortar containing the dry residue—more or less softened with fat—and the whole thoroughly blended to produce a uniform mixture. This final mixture was transferred by spatula to a paper Soxhlet thimble, covered with washed fiber asbestos wool, and a metal screen, and placed in the extraction tube of a large sized pyrex Soxhlet extractor. The chloroform necessary to operate the extraction was used in 6 or 7 portions to wash and rinse thoroughly the mortar, pestle, stirring rods, spatulas, and camels' hair brush used in mixing and transferring the mass to be extracted. These portions of chloroform were filtered successively through a small folded filter. The extraction with C. P. chloroform was continued for 72 hours. Usually after the first 24 hours a flask containing fresh solvent was introduced in place of the flask of solvent containing the extraction of the first 24 hours.

Cream: After evaporation of the solvents in the stream of filtered and heated air, and incorporation of the precipitate with the sand–Na_2SO_4 mixture, and before transferring to the extraction thimble, the mixture was flooded successively two or three times with C. P. chloroform. After thorough mixing with chloroform and subsequent settling, the chloroform was drained off by gentle suction each time into a flask and filtered through a small folded filter into an extraction flask and held until the 72-hour extraction was finished when it was incorporated with the remainder of the extraction solvents. From here on the cream residue-sand-Na_2SO_4 was treated exactly as has been described for the whole milk-skimmilk-buttermilk procedure.

Butter: Thirty grams of butter, weighed out as described, was mixed with the sand and anhydrous Na_2SO_4 (about one part butter to two parts Na_2SO_4), the mixture heated until the butter was melted, and the mixture flooded with chloroform and extracted as described for cream.

After the 72-hour extraction was completed, the exhausted thimble was removed from the extraction tube, which was used to receive the excess solvent, distilled off from the one to three extraction flasks containing different portions of the extract. The various portions of extract were combined and after the extract was reduced to the lowest safe volume it was transferred to an 800 cc. Kjeldahl flask and the extraction flask rinsed with chloroform. The Kjeldahl flask was partly immersed in hot water in a large beaker on an electric hot plate and the remaining solvent evaporated by a gentle current of filtered air.

Digestion: The digestion of the material from each extraction was accomplished with concentrated H_2SO_4 and HNO_3 and the volume of excess H_2SO_4 reduced with formaldehyde. A suitable amount of concentrated H_2SO_4, depending upon the amount of fat in the product, was added to the contents of the flask. From three to six glass beads were added to prevent bumping. The amount of H_2SO_4 was usually 25 cc. in case of whole milk, skim milk, and buttermilk, 80 cc. in the case of cream, and 60 cc. in the case of butter. The concentrated HNO_3 was added in very small portions or drop by drop from a separatory funnel, the rate depending upon the reaction. The larger the amount of fat to be digested, the more caution was necessary. No heat was applied to the flask until frothing had practically ceased. Then heat was applied slowly with a micro burner, and was gradually increased until the full flame of a regular Bunsen burner was used. After frothing had ceased, the HNO_3 was added at the rate of about 2 drops per second. Heat and speed of adding were carefully regulated to obtain a balance between overdue charring and excessive dilution.

Usually about 50 cc. of HNO_3 was necessary with whole milk, etc., and approximately 200 cc. with cream and butter. After the liquid in the flask

had become transparent, the addition of HNO_3 was discontinued, the nitric oxide fumes boiled off, and the addition of formaldhyde solution begun. Here again caution was necessary to add the solution fast enough so as not to take an excessive time to reduce the volume of H_2SO_4, and not so fast as to produce excessive charring on the side of the flask. The method is very satisfactory and rapid if a proper balance is maintained between temperature of heating and speed of dropping in formaldehyde solution.

The volume of H_2SO_4 was reduced to not more than 10 cc. When this had been accomplished the char in the flask was oxidized with concentrated HNO_3 added drop-wise while heating.

Phosphorus Determination: After the digestion was completed the H_2SO_4 content was approximately 10 cc. The contents were washed into a flask and made up to volume of 110 cc. For the determination of phosphorus the method of Woy (12) was chosen, because of the fact that phosphorus represents but 1.72% of the weight of the yellow precipitate after ignition to $P_2O_5 \cdot 24MoO_3$. The procedure was as follows: 50 cc. of the digested solutions were measured in duplicate into 400 cc. beakers, neutralized with NH_4OH, 25 cc. of 50% NH_4NO_3 and 20 cc. of 25% HNO_3 added, then heated until bubbling occurred and the required hot 3% ammonium molybdate run in from a separatory funnel in a thin stream, with continual shaking. In 10 to 15, min. the solution was clear and the yellow precipiate was ready to filter. After washing the precipitate with 5% ammonium nitrate 2 or 3 times, by decantation, the precipitate was dissolved in NH_4OH, reprecipitated by the addition of the necessary amount of ammonium nitrate, ammonium molybdate and HNO_3. The yellow precipitate is then filtered into a Gooch crucible, washed with 5% ammonium nitrate, ignited in the muffle at a temperature of 400–450° C. and weighed as $P_2O_5 \cdot 24MoO_3$. The phosphorus represents 1.72% of the weight of the precipitate.

RESULTS

Whole milk of 3.88 per cent fat content was separated and the resulting cream was churned and samples of each product collected for analyses. The separator slime was not collected separately but was washed into the skim milk fraction.

Each sample was extracted as described and the phosphorus content of the extract determined. This was multiplied by 25.00 to convert the phosphorus into phospholipids. Following are the results of the analyses.

The results are in agreement with those of Mohr, Brockmann, and Müller. The seeming lack of agreement in the values for cream, butter, and buttermilk, may be accounted for by the differences in distribution of amount of product due to the use of cream of 23 per cent fat content, whereas the fat content of that used in this work was 41 per cent.

TABLE 2

Amount and distribution of phospholipids of milk

PRODUCT	AMT. USED	FAT IN PRODUCT	PHOSPHOLIPIDS IN FAT	PHOSPHOLIPIDS IN PRODUCT	PHOSPHOLIPIDS IN PRODUCT		PERCENTAGE OF TOTAL PHOSPHOLIPIDS IN MILK	PERCENTAGE OF PHOSPHOLIPIDS IN CREAM
	gm.	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*per cent*	*per cent*
Whole milk (3.88)*	152,628	5,921.97	.0869	.0337	.....	51.44	100.00	.....
Skim milk	137,818	124.04	17.29	.0169	23.29	.....	45.28	.....
Cream (41.13)*	14,815	6,093.41	0.442	.1816	26.90	.....	52.30	.....
					Total	50.19		
Cream	.....	.....	.....	.....	.....	26.90	.....	.....
Buttermilk	8,017	155.53	9.378	.1819	14.58	.....	28.34	54.20
Butter	6,798	5,765.38	0.2207	.1872	12.72	.....	24.73	47.39
					Total	27.30		

* Per cent fat content of product.

TABLE 3

Phospholipid content of milk and milk products according to Mohr, Brockmann and Müller, Horrall, and the authors

	MOHR, BROCKMANN AND MÜLLER (13)	HORRALL (6)	AUTHORS
	per cent	*per cent*	*per cent*
Whole milk	.037	.0276	.0337
Skim milk	.0155	.0166	.0169
Cream	.1685 (23%)*	.155 (37.67%)*	.1816 (41%)*
Butter	.2060	.1685	.1819
Buttermilk	.1142	.1415	.1872
Separator slime	.....	.68	.....

* Per cent fat content of cream.

As stated heretofore the results previously published upon the phospholipid distribution in milk products were in error. This does not apply, however, to the results obtained in experiments wherein the fat was used in the analyses (articles II and III of the series). In these experiments it was shown that the amount of phospholipids in the fat extracted from representative samples of buttermilk by the Roese-Gottlieb method was 13.18 per cent (av.), and in that from skim milk was 16.36 per cent (av.), the actual value in any case being dependent upon the fat content of the product. Horrall obtained values of 13.91 and 19.66 per cent upon representative samples of skim milk and buttermilk, respectively.

CONCLUSION

The chemical nature of the phospholipids of milk has been discussed, their approximate solubilities given, and a tentative composite molecular weight established. In view of the solubilities given it is doubtful if extraction of phospholipids can be completed without the use of hot ethanol or the supplementary use of extraction with chloroform.

The molecular constitution of the phospholipids has been discussed and the molecular weight calculated as 775.76 on the basis of analyses of the phospholipid fractions. On this basis the factor for the conversion of phosphorus into milk phospholipids would be 25.00.

The distribution of the phospholipids among some of the products has been determined.

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INFECTIOUS BOVINE MASTITIS

REPORT ON A CONTROL PROGRAM BASED ON SEGREGATION OF INFECTED ANIMALS*

W. N. PLASTRIDGE, E. O. ANDERSON, F. J. WEIRETHER AND R. E. JOHNSON
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In a previous publication (1), a program for the control of infectious bovine mastitis was described and the results of preliminary observations presented. The plan involved systematic periodic examinations of the milk and segregation of affected animals. The segregation plan was started in one herd in 1930 and continued for a period of two years. Since 1932 data have been collected on six other herds. All animals were free from Bang's abortion disease and tuberculosis. It is the purpose of this paper to present briefly the results obtained up to the present time.

METHODS**

Individual quarter samples were collected aseptically from all milking animals in the six experimental herds at intervals of three months. The following determinations were made on each sample: Physical appearance, reaction to bromthymol blue, leucocyte count, microscopic examination of films prepared from incubated portions of the samples, and isolation and identification of streptococci, when present. A leucocyte count of 500,000 or more and the presence of *Streptococcus mastitidis* (Group A)*** were considered positive evidence of infectious mastitis. On the basis of the tests employed, the milking animals in six of the seven herds under observation were divided into one of the following groups:

Group I. Animals classed as negative.

Group II. Animals showing suspicious or positive evidence of mastitis due to causes other than streptococci (chiefly hemolytic staphylococci). Evidence of mastitis associated with staphylococci was usually transitory and was limited, with few exceptions, to an abnormally high leucocyte count.

Group III. Animals showing suspicious or positive evidence of mastitis due to streptococci other than *Streptococcus mastitidis* (Group A); usually

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* The control program was described before the 1934 (December) meeting of the North American Conference of Official Research Workers in Animal Diseases. The results reported herein were presented at the 1935 (December) meeting.

** For a description of methods employed, readers are referred to Bulletins 195 and 197 of the Storrs Agricultural Experiment Station, Storrs, Connecticut.

*** Identical with "Mastitis streptococcus Group 1" of Minett (2), and is apparently identical with *Streptococcus agalactiae* (Kitt, 1893).

Group B streptococci, which is apparently identical with "Mastitis streptococcus Group III" of Minett (2).

Group IV. Animals shedding *Streptococcus mastitidis* (Group A) without showing other evidence of mastitis.

Group V. Animals eliminating *Streptococcus mastitidis* (Group A) and showing other positive evidence of mastitis.

The animals were arranged in the barn and milked in the order suggested by the group classification.

RESULTS

The results obtained to date on seven experimental herds in which mastitis has been observed for periods of from one to six years are given here, briefly.

Herd F. Preliminary observations on the influence of segregating animals showing evidence of chronic streptococcal mastitis were made on Herd F, and were referred to in 1934 in Storrs Experiment Station Bulletin 197. A summarization of the data collected over a period of four years (1928 to 1932) is given in Table 1.

TABLE 1

Incidence of Streptococcal mastitis in Herd F.*

ANIMALS	BEFORE SEGREGATION		AFTER SEGREGATION	
	1928 to 1929	1929 to 1930	1930 to 1931	1931 to 1932
Total number	27	34	30	26
Had mastitis previously	3	7	4	1
Changed from negative to positive	6	3	1	0
Change from positive to negative	0	1	0	0
Positive animals removed	2	5	4	0
Negative animals removed	2	9	9	0
1st calf heifers	9	11	10	9
Total incidence, per cent	33.3	29.4	16.6	3.8
Incidence of new infection, per cent	22.2	8.8	3.3	0.0

**Streptococcus mastitidis* (Group A).

During the first 18 months no attempt was made to segregate animals affected with mastitis, with the result that during the first year the total incidence of infection with *Streptococcus mastitidis* (Group A) was 33.3 per cent and the incidence of new infection 22.2 per cent. Late in the second year the infected animals were placed at one end of the barn and milked last; five infected animals and nine streptococcus-free animals were sold and eleven first calf heifers added. During the second year the total incidence of affected animals was 29.4 per cent and the incidence of new infection decreased to 8.8 per cent.

The policy of segregation and gradual replacement of infected animals with first calf heifers was followed during the next two years. As a result the total incidence of infection was reduced from 29.4 per cent to 3.8 per cent. The incidence of new infection during the third year, or first year following segregation, was 3.3 per cent and was nil the following year. Further data were not secured, as the entire herd was disposed of in the summer of 1932.

Herd C. The average yearly incidence of streptococcal mastitis in Herd C for a period of four years preceding segregation is available for comparison with the incidence of the disease following adoption of the segregation program. Herd C differed from the others by being divided into four general sections according to breed. The results obtained in Herd C before and after segregation of affected animals are summarized in Table 2.

TABLE 2

Incidence of Streptococcal mastitis in herd C*

ANIMALS	BEFORE SEGREGATION	AFTER SEGREGATION	
	Yearly average for 4 year period preceding segregation	1 year 2/19/34–2/5/35	8 months 2/5/35–10/23/35
Total number	55	57	52
Had mastitis previously	14	11	6
Changed from negative to positive	7	2	1
Changed from positive to negative	0	2	1
Positive animals removed	7	3	1
Negative animals removed	7	12	8
1st calf heifers	18	16	10
Total incidence, per cent	38.2	22.8	13.5
Incidence of new infection, per cent	12.7	3.5	1.9

**Streptococcus mastitidis* (Group A).

During the four years preceding segregation the average yearly incidence of streptococcal mastitis was 38.2 per cent, and the average yearly incidence of new infection 12.7 per cent. During the first year of segregation the incidence of new infection decreased from the preceding yearly average of 12.7 per cent to 3.5 per cent, and the total incidence of infection decreased from the average of 38.2 per cent to 22.8 per cent. During the following eight months only one additional animal yielded laboratory evidence of streptococcal mastitis, and the total incidence of mastitis decreased to 13.5 per cent.

The reduction in total incidence of streptococcal mastitis from the average of 38.2 per cent before segregation to 13.5 per cent 20 months following adoption of the segregation program is attributed to three factors: (1) a marked reduction in the rate of spread as the result of segregating

infected animals, (2) apparent recovery of three animals, and (3) replacement of three positive animals by first calf heifers.

Herd G. The results obtained on Herd G are of particular interest because mastitis was a serious problem in this herd from the time the experimental use of bacterins was started in 1928 until after a program of segregation was started in the fall of 1933. The results of periodic examinations made during the two years preceding segregation have been summarized in Table 3 for comparison with the results obtained following segregation.

TABLE 3

Incidence of Streptococcal mastitis in herd G*

ANIMALS	BEFORE SEGREGATION		AFTER SEGREGATION	
	1931–32	1932–33	1933–34	1934–35
Total number	41	40	50	48
Had mastitis previously	12	4	7	0
Changed from negative to positive	4	5	0	1†
Changed from positive to negative	0	0	0	1†
Positive animals removed	12	2	7	1†
Negative animals removed	3	2	7	4
1st calf heifers	5	14	14	12
Total incidence, per cent	39.0	22.5	14.0	2.1
Incidence of new infection, per cent	9.8	12.5	0.0	2.1

**Streptococcus mastitidis* (Group A).

† This animal occupied quarters formerly used by positive group, yielded Group A streptococci on last test of lactation period, was negative on first test following calving and was sold shortly after calving.

During the year 1931–32 the total incidence of streptococcal mastitis in this herd was 39.0 per cent, and the incidence of new infection 9.8 per cent. Twelve animals that had been affected with chronic mastitis for several years were disposed of for economic reasons. However, four infected animals were left in the herd. The following year (1932–33) five instances of new infection accounted for a 12.5 per cent increase in infection. These five animals, together with the four previously infected ones, made a total incidence of 22.5 per cent. Two of the nine positive animals were sold.

The segregation program was begun in the fall of 1933 with seven positive animals in the herd at the start. These were placed at one end of the barn and milked last. Since no new infection occurred during the first year of segregation (1933–34), and as two positive animals were sold and 14 heifers added, the total incidence of mastitis decreased to 14.0 per cent. Since the program seemed to be progressing satisfactorily and a number of first calf

heifers were available for replacements, the owner disposed of the remaining seven positive animals.

At the beginning of the second year of the segregation program none of the animals showed evidence of infection with *Streptococcus mastitidis* (Group A). However, after the herd was rearranged, following disposal of the seven positive cows, negative animals were moved into the quarters formerly occupied by the positive group. One of these animals became infected with Group A streptococci and showed slight evidence of mastitis on a single test, but returned to negative on the following test. She was sold shortly thereafter. This animal accounts for the one instance of new infection and the single instance of recovery noted in the last column of Table 3. The total incidence of infection for the year 1934–35 was 2.1 per cent.

It is interesting to note that four of the five cases of new infection observed during the year 1932–33 were animals located next to or second from a positive animal.

Three instances of infection with Group B streptococci occurred in Herd G during the two year period from 1933 to 1935. One infected quarter gave abnormal milk for a period of several weeks immediately following calving. Thereafter the secretion from this quarter was normal in appearance and failed to yield either laboratory evidence of mastitis or Group B streptococci on subsequent tests. Evidence of mastitis in the other two animals infected with Group B streptococci was limited to an abnormally high leucocyte count. One of these yielded Group B streptococci on one test only; the other shed Group B streptococci for a period of about 15 months. The secretion from this animal was normal in appearance and repeatedly gave a normal reaction to the bromthymol blue test.

Herd Ch. Periodic examinations were made on Herd Ch for a period of 15 months. In as much as the segregation program was not carried out as recommended, the results obtained on this herd are of value principally for comparison with the results obtained on herds in which segregation was practiced. While the animals placed in Group 5 were later removed to a position near the end of the milking string, this was not done consistently and several months usually elapsed between the time the animals were classified and the time Group 5 animals were moved to the end of the line. The results obtained on Herd Ch are summarized in the first column of Table 4.

Six animals were classified as positive in the first test. During the 15 months' period of observation five additional animals became infected, with the result that the total incidence of mastitis was 24.4 per cent and the incidence of new infection 11.1 per cent. Four of the five instances of new infection were apparently traceable to two positive animals which were located near the head of the milking string and which were not removed as

TABLE 4

Incidence of Streptococcal mastitis in four other experimental herds*

ANIMALS	NOT SEGREGATED	SEGREGATED	SEGREGATED	NEGATIVE HERD
	Herd Ch. (15 months)	Herd Gr. (12 months)	Herd H. (12 months)	Herd O. F. (3 years)
Total number	45	59	61	11
Had mastitis on first test	6	13	9	0
Changed from *negative* to *positive*	5[a]	4[b]	3[c]	0
Changed form *positive* to *negative*	1	0	0	0
Positive animals removed	4	5	3	0
Negative animals removed	5	9	6	1
1st calf heifers added	13	25	17	3
Total incidence, per cent	24.4	28.8	19.7	0.0
Incidence of new infection, per cent	11.1	6.8	4.9	0.0

* *Streptococcus mastitidis* (Group A).

[a] Four of the five instances of new infection were animals located next to previously affected animals which had not been segregated.

[b] All four instances of new infection were animals located next to the positive group (Group 5).

[c] In two instances infection followed injury to a teat. The third animal was located next to the positive group (Group 5).

recommended at the time of the preceding test. The fifth animal was located next to a positive cow near the end of the milking string.

Herd Gr. The segregation program was started in Herd Gr in July, 1934. Animals were arranged in the barn and milked in the order suggested by the grouping. The results obtained on Herd Gr are summarized in the second column of Table 4.

Thirteen animals were placed in Group 5 at the time of the first test. During the year four animals became infected. The position of the newly infected animals in this herd is of particular interest. On the second test, one new positive was found. This animal was located next to the first animal in Group 5. On the third test two new positives were found. One of these was located next to the first animal in Group 5, and the second next to the other newly infected animal. The single newly infected animal, identified as such in the fourth test, was also located next to Group 5. The incidence of new infection for the twelve months' period of observation was 6.8 per cent, or at least half of what might be expected if the positive animals had not been segregated.

The results obtained with Herd Gr suggest three important factors that must be considered in the control of infectious streptococcal mastitis: (1) that grouping the positive animals at one end of the barn and milking them

in the order suggested by this grouping greatly reduces, but does not entirely prevent spread of infection, (2) that apparently infection may be transmitted to negative animals by contact with floors that have been infected by carrier animals, and (3) that isolation of the positive animals, rather than placing them at the end of the milking string, is necessary to prevent completely the spread of infectious streptococcal mastitis.

Herd H. The milking animals in Herd H were examined for the first time in November, 1934. The segregation program was started as indicated by the results of the first test. The data secured are summarized in the third column of Table 4.

At the outset nine animals were placed in Group 5. During the year three additional animals became infected with *Streptococcus mastitidis* (Group A). In two instances infection was preceded by injury to the teat of the affected quarter. The location and order of milking of these animals following injury is not known. The third animal that became infected was located next to the first animal in Group 5. During the year the total incidence of mastitis due to Group A streptococci was 19.7 per cent, and the incidence of new infection was 4.9 per cent.

Herd O. F. The results obtained with Herd O. F. are summarized in the fourth column of Table 4. This herd is of particular interest, as it was started in 1932 from eight pregnant heifers purchased from a herd which was accredited free from Bang's abortion disease and tuberculosis. Samples were collected from all milking animals and examined at intervals of one month. Several of the cows are now in their third lactation. During the three year period of observation over 500 quarter samples were examined without finding a single instance of infection with either *Streptococcus mastitidis* (Group A) or with Group B streptococci. Saprophytic streptococci which were readily differentiated from *Streptococcus mastitidis* (Group A) and Group B streptococci, were obtained from 15 samples. None of these samples contained an abnormal number of leucocytes or showed other evidence of mastitis.

DISCUSSION

While numerous species of bacteria have been found to be associated with bovine mastitis, streptococci have been reported most frequently as the common cause of the disease. It is also evident from the literature on this subject that more than one species of streptococci may cause mastitis. However, the work of Jones (3) in 1918 indicated that the majority of streptococci associated with mastitis possessed similar characteristics. More recently numerous reports have shown that the common cause of infectious bovine mastitis is a fairly well defined species of Streptococcus (4, 5, 6, 7, 8, 9, 10 and 11). The recognition of a specific organism as the common causative agent in infectious bovine mastitis offers a definite basis for a

program of control based on prevention by segregation of animals harboring this organism.

The advisability of segregating animals affected with mastitis has been recognized since the early work of Franck (12) in 1875 and Nocard and Mollereau (13) in 1887. A decrease in incidence of mastitis in four herds over a two year period following a program of isolating cows affected with mastitis and careful attention to dairy hygiene, was observed by Hardenberg and Schlotthauer (14). Udall and Johnson (15) in 1930 and 1931 advised placing all affected animals, as determined by physical examination of the udder and reaction of the udder secretion to the bromthymol blue test, in a single group, to prevent spread of infection to mastitis-free animals. More recently isolation of animals based on the results of cultural tests for the detection of streptococci in the udder secretion has been recommended by Seelemann (16), Steck, Bachmann, Kaestli and Gygax (17), Hucker (18), Minett, Stableforth and Edwards (19), and Plastridge, Anderson, Brigham and Spaulding (7).

Evidence that herds free from *Streptococcus agalactiae,* referred to in our reports as *Streptococcus mastitidis* (Group A) may be established by a program of segregation and gradual replacement of infected animals with first calf heifers was presented by Minett, Stableforth and Edwards (19) in 1933, and by Plastridge, Anderson, White and Rettger (1) in 1934. Recently Stableforth, Edwards and Minett (20) have described the results of further observations on six herds in which contagious streptococcal mastitis was reduced and in some instances eliminated by segregation and disposal of infected animals.

Results obtained in six herds by use of the segregation program described by us (1) in 1934 are presented here. Observations made on these herds showed that the annual rate of spread was reduced from 50 to 100 per cent by use of the plan. In one herd (Herd G) infection with *Streptococcus mastitidis* (Group A) was completely eliminated by gradual replacement of infected animals with first calf heifers.

With few exceptions, the limited number of instances of new infection occurred in animals located in the barn next to the group infected with streptococci (Group 5). As the negative animals were milked first, it appears that in these instances infection spread to the negative animals by contact with the floor or litter contaminated by the infected animals.

The results obtained on a herd recruited from pregnant heifers (Herd O. F.) are of particular interest, as tests made at monthly intervals since the herd was started in 1932 show that the animals have remained free from *Streptococcus mastitidis* (Group A) up to and including the time of the last test which was made January 20, 1936. This finding is in accord with that reported by Seelemann (16), who observed that several self-contained herds were entirely free of the "galt" streptococcus, and that the herds remained so unless infected animals were introduced.

SUMMARY

Observations on the incidence of streptococcal mastitis in seven experimental herds over periods of from one to six years are described. Data collected before and after the adoption of a program of segregation based on periodic examinations (chiefly bacteriological) and segregation of animals shedding streptococci identified as *Streptococcus mastitidis* (Group A)*, are presented. The results obtained indicate that:

1. The annual rate of spread of infectious streptococcal mastitis in infected herds may be reduced from 50 to 100 per cent by the use of the segregation plan described in this paper and in a previous publication (1).

2. While the rate of spread of infection is materially reduced by segregating infected animals at one end of the milking string and milking them last, complete separation is necessary to entirely prevent the spread of infection.

3. Herds free from *Streptococcus mastitidis* (Group A) may be established by segregation of the normal animals, disposal of infected individuals, and replacement by first calf heifers that have not been exposed to infection following parturition.

The results presented support the opinion that it is possible to establish and maintain a herd free from the organism generally recognized as *Streptococcus agalactiae*.

The writers are indebted to Professors L. F. Rettger and G. C. White for valuable suggestions in planning the work described herein.

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PYGOPAGUS PARASITIC BOVINE TWINS INVOLVING THE UDDER*

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The frequency of twinning in cattle is rather low. Johansson (1932) in a recent survey of the literature, observed a frequency of 1.88 per cent in dairy cattle in a tabulation of 243,016 births and only 0.44 per cent in beef cattle in a tabulation of 748,855 births. Still more rare is the appearance of double monsters or conjoined twins. Johansson presented a figure of such twins preserved in the museum of the New York State Veterinary College which were joined posteriorly of the region of the diaphragm. The

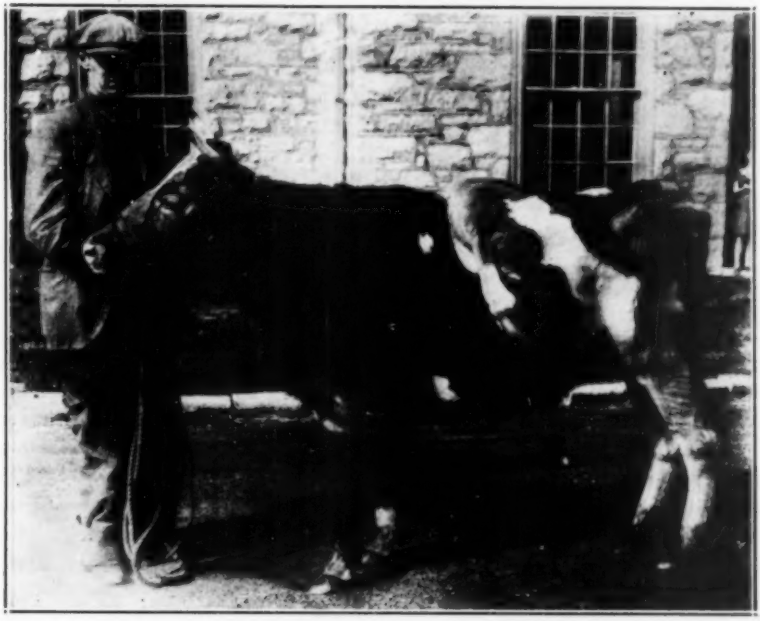

FIG. 1. *Pygopagus Parasitic Bovine Twins.* The above picture shows a rare anomaly of twinning in which one member is completely represented and normal, whereas the other, a parasite, is smaller in size and in this case represented by certain posterior sections of the body.

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FIG. 2. *View of the Parasitic Udder.* In addition to the complete udder and teats there may be seen a leg and tail. Two hip points may be palpated in front of the tail. It is suggested that supernumerary mammary glands far removed from the location of the embryonic mammary line may arise as a parasitic gland from a twin which later degenerated.

anterior and free parts displayed a pronounced reversal of symmetry in color patterns. He also reported that Krönig (1924) had described 10 cases of two-headed calves and one case of "Siamese" cattle twins.

A very rare anomaly of twinning is the presence of one member which is completely represented and normal and the other, called the *parasite*, which is smaller in size and more or less imperfectly developed. The object of the present paper is to present a photograph and brief description of such a case.

The animal is owned by Mr. C. C. Burkett, of Missouri Valley, Iowa, who kindly permitted the photographs to be taken and gave the following information about the case. The animal was reported to have been dropped by a registered Guernsey cow at Persia, Iowa, in February, 1932. At the time examined (April 5, 1935) she was just over two years old. She was reported to come into estrum quite regularly although had not been bred.

As will be seen from the figures, the parasite consisted externally of a leg, tail, hip bones and a complete udder with four teats. Two of the teats were about one inch long, while the other two were quite rudimentary.

Rectal palpation disclosed another leg internally with part of the backbones and other structures as well.

The writer's special interest was in the mammary gland. Many cases of supernumerary teats have been reported in the literature. Gifford (1934) recently presented a review and added a large number of records collected in Missouri. These cases in cattle consist of additional teats and glands at the rear of or between the normal teats. Gifford found three animals with four supernumeraries at the rear of the normal teats in a population of 3582 cows examined. However, these teats were located along the embryonic mammary line (see Turner, 1930) and their position may be considered normal. The writer is not familiar with reports of either individual glands or of entire udders in abnormal locations in cattle.

In man, supernumerary nipples and glands have been reported in many positions far removed from the location of the embryonic mammary line—the thigh, the neck and back (see Deaver and McFarland, 1917, or Fitzwilliams, 1929). It has been difficult to account for such cases on the theory that they arise from proliferations of the mammary line. The case here reported suggests the possibility that such glands might arise as the result of the development of a parasitic twin, the structures of which were largely if not entirely absent with the exception of the mammary gland or glands. In other words, the presence of mammary glands in positions far removed from the location of the embryonic mammary line may be due to the fusion of the mammary gland from the parasitic twin with the normal twin, with the loss of all other parts of the parasite.

According to this theory, the supernumerary teats and glands might be classes as arising from the excessive proliferation of the mammary line or in rare cases as a parasite gland from a twin which later degenerated.

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ACCUMULATION OF PROTEIN IN THE FOAM OF SKIMMILK

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INTRODUCTION

Gibbs, from theoretical considerations, made the deduction that the concentration of the solute at the surface might differ from that in the body of a solution, and that solutes which lower the surface energy, concentrate at the surface. This deduction was later verified experimentally. A reduction in surface tension is now accepted as evidence of the accumulation of materials at the surface of the solution. Direct evidence of accumulation is obtained by causing the solution to foam and analyzing separately the foam and the liquid beneath it.

Gibbs' deductions were based on reversible systems. Proteins lower the surface tension of their solutions and accumulate at the surface, but the process is usually not reversible, and the layer at the surface may be several molecules thick, as shown by Plateau (7), Ramsden (9), Shorter (10), and others. This accounts for the great accumulation of protein at the surface.

Siedel and Hesse (12) analyzed milk and its foam and found that protein accumulated in the foam. A summary of some of their data is given below:

	Milk	*Foam*
Acidity (Thörner)	15.97	18.76
Viscosity (Time of flow, min.)	69.5	75.9
Ash %	0.749	0.775
Calcium %	0.166	0.181
Dry matter %	8.68	8.99
Protein %	3.11	3.49
Specific gravity	1.0345	1.0356

The increase in total solids in the foam is almost quantitatively accounted for by the increase in protein. The increase in protein in the foam probably accounts for the increase in viscosity, specific gravity, and titratable acidity. The accumulation of calcium might be associated with an accumulation of casein.

Siedel (11) found that foam on the skimmilk from the separator was prevented by churning the milk before separation.

Rahn and Sharp (8) state that after six consecutive foamings and removals of the foam from whole milk, the liquid remaining foamed only slightly. This experiment was interpreted as indicating that the foam-producing material had been removed. The breaking of the foam at the

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end of churning and the failure of the skimmilk to foam in Siedel's experiments were interpreted as indicating that the foaming material had been irreversibly coagulated and none was left to cause foaming. Ansbacher, Flanigan and Supplee (1) eluted a protein fraction from casein which showed marked foaming properties when added to water and which was almost completely removed in the foam.

The experiments of Siedel and Hesse show that the protein content of the foam is 0.4 per cent higher than that of the milk from which it was obtained. This is an appreciable difference in protein content. If a specific protein is concentrated in the foam to the exclusion of the others, it should be a simple matter to concentrate, separate and identify it by repeatedly foaming the milk and removing the foam.

EXPERIMENTAL

Skimmilk was selected because the foam-breaking effect of the fat would not be a complicating factor. It was found that one of the best ways to produce foam on an appreciable volume of milk was to run it through the separator. Of the separators available the one which produced the most foam was selected. About 80 pounds of milk were separated at 32° C. The cream outlet of the separator bowl was then plugged and the skimmilk was rerun through the separator and was collected in a small vat with gate valve at the bottom. After standing for 5 minutes the liquid beneath the foam was drawn off and again run through the separator. This process of passing through the separator and removal of the foam was repeated from 10 to 16 times in various experiments. At intervals, samples of the milk, foam, and liquid beneath the foam were taken for analysis. Every effort was made to avoid errors due to evaporation. The vat and vessels containing the foam and milk fractions were kept covered with wet cloths.

Some of the data obtained in two series of experiments are reported. In one experiment the milk was passed through the separator 16 times at 32° C. In the other the milk was first separated at 32° C. and was then separated 13 times at 10° C.

The repeated foaming and removal of the foam did not exhaust the foam-producing capacity of the skimmilk; in fact, the foam became more stable, particularly during the first few times the milk was separated. The volumes of foam on the milk 5 minutes after separation at 32° C. are shown in Table I. This table indicates that the material which accumulated in the foam is almost entirely protein. This is in agreement with the results obtained by Siedel and Hesse.

Hekma and Brouwer (3) concluded that the separator slime came from collapsed foam cells. Van Slyke and Bosworth (14) ran milk through the separator 18 times. After the first run the composition of the slime was approximately constant. They make no comment about foaming proper-

TABLE I

Adsorption of protein in the foam of skimmilk at 32° C.

NUMBER OF TIMES THROUGH SEPARATOR	MILK BEFORE SEPARATION		MILK BENEATH FOAM AFTER SEPARATION		FOAM		AMOUNT ACCUMULATED IN FOAM		VOLUME OF FOAM	SLIME ON OUTER BOWL COVERING	
	Protein	Total solids	Protein	Total solids	Protein	Total solids	Protein	Total solids			
	%	%	%	%	%	%	%	%	%	*gram*	%
1	3.17	8.58	3.14	8.79	3.77	9.39	0.63	0.60	27	21	0.034
2	3.14	8.79	3.14	8.82	3.68	9.47	0.54	0.65	39	8	0.016
6	3.12	8.90	3.08	8.88	3.70	9.36	0.62	0.48	42	4	0.010
8	3.02	8.81	3.00	8.83	3.68	9.56	0.68	0.73	43	4	0.010
16	2.80	8.26	2.74	8.34	2.98	8.50	0.24	0.16	50	12*	0.111
Collected foam 9 to 16 re-run	3.15	8.79	2.96	8.70	3.07	8.71	0.11	0.01	45	69	0.945

Slime from re-run foam 24.9% total solids, 20.1% protein.

* High due to souring.

ties. Table I of the present study shows that, after the first time through the separator, the amount of slime was relatively small. At the end of the experiment, however, some of the collected foam was rerun and a very large amount of slime was obtained. It is probable that the slime obtained when the foam was rerun was due to souring.

Milk was separated 13 times at 10° C., and the protein fractions were determined on every third run. The casein was precipitated with acetic acid and the nitrogen content of the resulting whey was determined. This value subtracted from the total nitrogen gave the casein by difference. An aliquot of the whey filtrate was heated to boiling at pH 5.0, cooled, and filtered. A nitrogen determination of the filtrate gave the non-heat-coagulable protein, and this value subtracted from the protein in the whey filtrate gave the heat-coagulable protein. The results obtained are presented in Table II.

Table II indicates that no appreciable change in the relative amounts of the main protein fractions of milk is produced by repeated foaming and removal of the foam. These results indicate that the proteins are concentrated in the foam in the same proportion in which they occur in milk. No indication was found that the foaming power of the skimmilk was exhausted. The percentage volume of foam was practically constant at 60 per cent after each separation in Table II.

Lots of skimmilk were reseparated 16 times at 5° and at 50° C., the foam being removed after each separation. No diminution in foaming properties was observed.

The air cells are hardly small enough to explain the marked accumulation of protein in the foam on the basis of the formation of a monomolecular layer of proteins of the albumin type. Gorter and Grendel (2) and Neurath (6) have found that 1 mg. of proteins of this type will cover about 1 square meter of surface with a complete layer one molecule thick. Leviton and Leighton (5) found that 1.7 mgm. of protein was required to cover 1 square meter of milk serum surface. With accumulations running as high as 600 mg. per 100 cc., one would have to assume 350 to 600 square meters of foam surface from 100 cc. of foam liquid.

The material which lowers the surface energy the most should be present in the outer surface layer almost to the exclusion of other materials, provided no other factor comes into play. The data in Table II show that the proteins accumulate in the foam in the proportion in which they occur in milk. No satisfactory explanation for this behavior has been found. It is possible that some specific material might form the final outer layer and our analytical procedures were too crude to reveal it. Possibly each of the proteins in milk lowers the surface energy to nearly enough the same degree that each would be crowded toward the surface. Thus the proteins might concentrate in the surface in the proportion in which they occur in milk. It is

TABLE II

Protein fractions in skimmilk after repeated separation at 10° C. (50° F.)

NUMBER OF TIMES THROUGH SEPARATOR	TOTAL PROTEIN	CASEIN		HEAT COAGULATABLE		NON-HEAT COAGULATABLE	
		Total milk	Total protein	Total milk	Total protein	Total milk	Total protein
	%	%	%	%	%	%	%
Milk before separation							
2	3.89	3.05	78.4	0.40	10.3	0.44	11.3
5	3.69	2.89	78.3	0.37	10.0	0.43	11.7
8	3.57	2.77	77.6	0.37	10.4	0.43	12.0
11	3.48	2.71	77.9	0.34	9.8	0.43	12.3
14	3.32	2.56	77.1	0.32	9.6	0.44	13.3
Collected foam re-run	3.95	3.17	80.2	0.37	9.4	0.41	10.4
Milk beneath foam after separation							
2	3.82	3.01	78.8	0.30	7.9	0.51	13.3
5	3.69	2.90	78.6	0.36	9.8	0.43	11.6
8	3.56	2.77	77.8	0.35	9.8	0.44	12.4
11	3.47	2.69	77.5	0.37	10.7	0.41	11.8
14	3.32	2.57	77.4	0.32	9.6	0.43	13.0
Collected foam re-run	3.83	3.02	78.9	0.38	9.9	0.43	11.2
Foam							
2	4.19	3.35	79.9	0.40	9.6	0.44	10.5
5	4.00	3.20	80.0	0.35	8.8	0.45	11.2
8	3.82	3.00	78.5	0.39	10.2	0.43	11.3
11	3.63	2.84	78.3	0.36	9.9	0.43	11.8
14	3.44	2.67	77.6	0.36	10.5	0.41	11.9
Collected foam re-run	4.10	3.27	79.8	0.37	9.0	0.46	11.2

further possible that the dynamic surface created by the foaming more or less "fixes" all protein material that happens to be present at the surface, at the instant of its formation. The proteins would therefore naturally be fixed or surface altered in the same proportion in which they occur in milk.

It was stated in the book by Rahn and Sharp (8) that the foaming ability of whole milk was greatly reduced by whipping, removing the foam, and rewhipping the liquid remaining. The decrease in foaming ability of the milk was explained as due to the removal of the foam-producing substance. The foaming was greatly reduced after six re-whippings. This behavior of whole milk is not in agreement with the experiments reported here on skimmilk. Therefore similar foaming experiments with whole milk were made.

Whole milk was repeatedly whipped and the foam removed in a cold room at 2° C., without any diminution of the foam. Whole milk was run through a clarifier 16 times in several series of experiments, some of which

were carried out at 10° C. and others at 32° C., without any evidence of diminution of the foam. When whole milk was whipped in the laboratory after previous cooling with ice water, and the foam allowed to drain in a cylinder in the laboratory, and this procedure was repeated several times, a diminution in the foaming was observed.

These experiments with whole milk indicate that the decrease in foaming is not due to the removal of the foaming material but to the progressive liberation of a foam-breaking agent. The foam-breaking agent is liberated from the fat by the agitation. The decrease in foaming is probably similar to that observed when the cream breaks at the end of churning. Apparently the whipping must produce churning to reduce the foaming of whole milk.

Van Dam (13) and Leviton and Leighton (5) have shown and discussed the foam-breaking effect of small amounts of milk fat. Siedel (11) has shown that the foaming of skimmilk from the separator can be prevented by churning the milk for one hour before it is separated. Separating the milk, churning the skimmilk and then recombining the churned skimmilk and the unchurned cream did not prevent the foaming of the skimmilk when the reconstituted milk was reseparated. Churning the milk with the fat present was necessary to prevent foaming.

An experiment was made in which milk was churned for three hours. The temperature of the milk at the beginning of the experiment was 12° C. At the end of two hours it had risen to 14.5° C., and when the experiment was stopped the temperature was 19° C. Samples of the churned milk were removed at 15 minute intervals, and portions were separated at both 10° and 32° C., and the amount of foam on the skimmilk measured. The results obtained are expressed graphically in Figure 1. The volume of foam obtained on the skimmilk decreased progressively with churning, up to 1½ hours. From then on the amount of foam increased progressively. The decrease in the amount of foam on the skimmilk during the first hour and a half of churning is in agreement with the observation of Siedel. The later increase, due to prolonged churning, can possibly be accounted for by further changes in the material adsorbed at the air-liquid surface. It is possible that the increased foaming after prolonged churning was due to lipase activity. Considerable lipolysis occurs when raw milk is shaken, and this usually leads to pronounced foaming. This effect is particularly marked with milk and cream from cows in advanced lactation (4).

Buttermilk ordinarily foams less than skimmilk. The failure of buttermilk to foam is probably due to the presence of fat or fat-like material liberated from the fat globules, which enters the air-liquid interface and acts as a foam breaker, thus preventing the functioning of the natural foam-forming materials which are still present in the buttermilk. This was demonstrated by churning raw sweet cream and by comparing the foaming

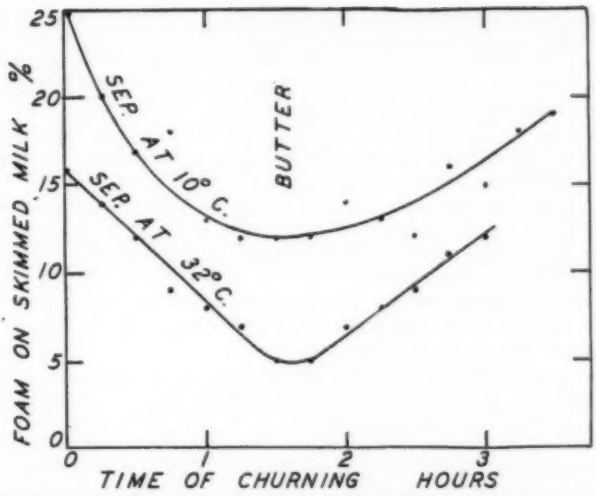

FIG. 1. Volume of foam on skimmilk, as influenced by the churning of whole milk previous to separation at 10° and 32° C.

properties of the buttermilk before and after separation and extraction with petroleum ether. The buttermilk which had been extracted with petroleum ether foamed readily at 5° and at 50° C. and appreciably at 25° C., while the natural buttermilk foamed hardly at all and the small amount of coarse foam formed soon collapsed.

CONCLUSIONS

1. Foaming and the removal of the foam, when repeated sixteen times, did not decrease the foaming capacity of skimmilk at 5°, 10°, 32°, and 50° C.

2. Protein accumulated in the foam of skimmilk to the extent of 0.12 to 0.68 per cent.

3. The relative amounts of the major protein fractions of skimmilk were not changed by repeated foaming. This indicates no preferential accumulation of any major protein fraction in the foam.

4. No decrease in the foaming properties of whole milk, due to repeated foaming, was observed unless churning occurred.

5. The foaming capacity of skimmilk separated from churned whole milk decreased progressively with the time of churning, until butter was formed.

6. The failure of buttermilk to foam readily is not due to the absence of foam-forming substances, but to the presence of foam-breaking substances.

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COMPARISON OF THE FEEDING VALUE OF STEAM DRIED AND FLAME DRIED MENHADEN FISH MEAL

M. H. BERRY[1] AND J. R. MANNING[2]

INTRODUCTION

Only in recent years has fish meal of a quality suitable for animal feeding become available upon the market in any considerable quantity. Considerable interest is now being shown in the use of this feed as a high protein supplement in animal feeding. Fish meal is also a valuable source of vitamins and minerals.

Investigations have shown that fish meals are superior to some meat by-products feeds in general feeding value and as sources of protein (1). In feeding trials with dairy cows in which fish meal has been used as a protein concentrate, it has compared very favorably with cottonseed, soybean and linseed oil meals (2), (3), (4), (5), (6). In rations for calves and young stock, fish meal has proved very satisfactory as a source of protein when compared with such protein supplements as linseed oil meal, tankage and blood flour. (5), (7).

METHODS OF MANUFACTURE

There are several widely differing processes of drying fish meals. The heat for drying may be supplied either by direct flame or by steam and the resulting products correspondingly termed "flame dried" or "steam dried" fish meal.

Daniel and McCollum (1) have shown in a series of experiments with rats, that vacuum or steam dried meals are superior to flame dried meals in feeding qualities. The findings of other investigators, (8), (9), (10) working with small animals and poultry, substantiate these statements and offer additional information.

At present, very little, if any, data are available showing the relative feeding value for large animals of fish meals produced by either of these drying methods.

It is the purpose of this paper to report the results of two feeding trials with growing heifers comparing the feeding value of flame dried and steam dried menhaden fish meal.

EXPERIMENTAL

Two separate feeding trials, each 120 days in length, were conducted, using animals from the Maryland Experiment Station herd. The work was started during the winter of 1934–35 and repeated the winter of 1935–36.

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The same general plan was used in conducting both trials. In both trials, the heifers were divided into two groups as similar as possible with respect to breed, age, live weight and general body condition. In Trial I, there were eight heifers per group. In Trial II, there were nine heifers in each group. Each group was quartered and fed in a large box stall in a shed. They were turned out daily for exercise, when the weather was favorable.

Group feeding was practiced throughout both trials. Six pounds of clover hay (U. S. No. 2 grade), and four pounds of a grain ration composed of two parts ground yellow corn, one part wheat bran and one part fish meal were fed daily per heifer. Fish meal, fed at the rate of one pound per day per heifer, made up 10 per cent of the total daily ration fed. Salt was kept before the animals in their feed troughs. Fresh water was accessible at all times.

Individual weights were taken for three consecutive days at the beginning and again at the end of the experimental periods and the average of the three weights taken as the initial and the final weight per heifer. An individual weight was also taken at the end of the first, second and third months.

COMPOSITION OF MEALS

Table I gives the analyses of the fish meals used in both feeding trials. In Trial I, two different lots of each meal were fed during the feeding period. In Trial II, one lot of the same meal was used throughout the experiment.

TABLE I

Analyses of menhaden fish meals used in feeding trials

	TRIAL I (1934–35)				TRIAL II (1935–36)	
	Steam dried		Flame dried		Steam dried	Flame dried
	Lot I	*Lot II*	*Lot I*	*Lot II*		
Protein (N×6.25)	62.50	63.50	60.12	62.81	61.54	60.57
Moisture	7.60	7.14	6.48	6.88	9.14	9.28
Ash	19.37	14.64	19.67	19.55	17.34	19.24
Ether Extract	3.61	5.71	4.63	6.62	10.63	9.91
Undetermined	6.92	9.01	9.10	4.14	1.35	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

It will be noted that the ether extract values are especially low and the percentage of undetermined constituents unsually high in the meals fed in Trial I. The fat content of menhaden fish meal ranges normally from 8 to possibly 12 per cent. It is believed that the low ether extract values of the

meals fed in Trial I are due to the fact that during storage a considerable proportion of this fat became oxidized and hence was not extractable by ordinary laboratory procedure. The samples of the meals fed in Trial I were not taken until after the close of the feeding trial. The samples of the meals fed in Trial II were taken during the feeding period and the analyses are therefore a true indication of their composition at the time they were fed.

DISCUSSION OF RESULTS

Complete results of the two feeding trials are reported in Table II.

It will be noted that in both trials the flame dried group made slightly larger average daily gains in weight than the steam dried group. This difference, however, is not significant.

At the conclusion of the first feeding trial, conducted during 1934–35 there was a decided difference in general body condition and appearance between the two groups of heifers, the advantage being in favor of the group fed the flame dried fish meal. The group fed the flame dried product was in a a fine condition of flesh and presented a very smooth, thrifty appearance throughout the trial. The heifers in the group receiving the steam dried fish meal were in a poor state of flesh at the end of the trial and presented a very rough, unthrifty appearance. No definite reason could be ascertained for this lack of flesh and general unthrifty appearance.

As it seemed best to secure additional data, the trial was repeated during the winter of 1935–36.

The growth results secured in Trial II were practically the same as those secured in Trial I although the heifers were slightly older. The rough,

TABLE II
Tabulation of results
All weights expressed in pounds

	TRIAL I		TRIAL II	
	Steam dried group	Flame dried group	Steam dried group	Flame dried group
Number of heifers per group	8	8	9	9
Number of days on trial	120	120	120	120
Av. intial age per heifer (months)	11.47	10.96	14.33	13.80
Av. intial weight per heifer	434.37	434.50	522.77	523.22
Av. final weight per heifer	548.12	558.87	632.11	647.33
Av. total gain per heifer	113.75	124.37	109.34	124.11
Av. daily gain per heifer	.94	1.03	.91	1.03
Daily ration fed per heifer				
Fish meal	1	1	1	1
Bran	1	1	1	1
Corn meal	2	2	2	2
Hay (U. S. #2 clover)	6	6	6	6
Total	10	10	10	10

TABLE III

Individual weight results—Trial I

Steam Dried Group

(All weights expressed in pounds)

HEIFER	INITIAL WEIGHTS				1ST MO.	2ND MO.	3RD MO.	FINAL WEIGHTS (4TH MONTH 120 DAYS)				GAIN IN WEIGHT	AV. DAILY GAIN
	1st day	2nd day	3rd day	Average				1st day	2nd day	3rd day	Average		
508	470	477	480	475	510	565	595	620	630	640	630	155	1.29
511	440	445	452	445	474	513	548	565	560	567	564	119	.99
512	405	407	409	407	425	435	465	481	483	482	482	75	.62
517	455	453	457	455	517	550	590	627	628	628	628	173	1.44
519	426	423	422	423	446	470	505	510	511	515	512	89	.74
522	385	387	389	387	405	400	430	452	450	460	454	67	.55
524	370	370	371	370	380	410	458	492	500	492	494	124	1.03
534	510	515	515	513	535	550	587	621	624	620	621	108	.90
Flame Dried Group													
505	483	495	500	492	549	588	610	625	630	637	630	138	1.15
507	492	496	498	495	560	580	595	627	625	630	627	132	1.10
513	437	441	446	441	474	505	535	548	545	555	549	108	.90
514	415	410	408	411	447	475	505	525	420	538	527	116	.96
518	465	463	464	464	535	562	590	591	598	605	598	134	1.11
520	410	410	407	409	440	485	518	560	550	552	554	145	1.20
521	342	342	343	342	361	390	407	409	415	413	412	70	.58
523	420	422	425	422	461	510	545	570	575	578	574	152	1.26

TABLE III

Individual weight results—Trial II

Steam Dried Group

(All weights expressed in pounds)

HEIFER	INITIAL WEIGHTS				1ST MO.	2ND MO.	3RD MO.	FINAL WEIGHTS (4TH MONTH 120 DAYS)				GAIN IN WEIGHT	AV. DAILY GAIN
	1st day	2nd day	3rd day	Average				1st day	2nd day	3rd day	Average		
519	726	740	734	733	750	800	860	891	882	895	889	156	1.30
525	640	635	635	636	643	665	680	680	681	675	678	42	.35
527	601	612	610	607	607	610	665	661	672	664	665	58	.48
529	680	692	696	689	711	745	773	785	785	774	781	92	.76
531	503	505	511	506	515	535	590	619	620	614	617	111	.92
532	458	462	460	460	496	519	570	611	606	610	609	149	1.24
536	423	422	426	423	455	475	502	532	537	532	533	110	.91
538	330	326	326	327	376	416	452	467	471	472	470	143	1.19
539	325	323	325	324	355	395	424	445	453	443	447	123	1.02
Flame Dried Group													
521	653	666	663	660	650	680	678	725	726	714	721	61	.50
524	702	711	714	709	755	831	848	900	897	895	897	188	1.56
528	583	580	590	584	614	642	660	696	697	690	694	110	.91
530	659	660	655	658	665	705	730	778	777	775	776	118	.98
533	535	537	536	536	545	560	581	615	625	620	620	84	.70
535	428	434	436	432	455	470	510	518	517	517	517	85	.70
537	443	440	444	442	486	523	565	587	581	590	586	144	1.20
540	325	320	328	324	375	416	455	491	494	495	493	169	1.40
541	367	357	368	364	415	457	483	518	520	528	522	158	1.31

unthrifty appearance and lack of condition that was evident with the heifers in the steam dried group in Trial I did not appear in Trial II. No differences in the condition of the animals on the two different meals in the second trial were apparent to the eye.

No difficulty was experienced in getting the heifers to eat readily the grain ration containing fish meal.

SUMMARY AND CONCLUSIONS

The comparative feeding value of steam dried and flame dried menhaden fish meal was determined by conducting two separate 120-day feeding trials during two successive years with two groups of yearling heifers. Fish meal made up 10 per cent of the total ration fed daily per heifer.

There was no significant difference between the two meals in either trial in respect to gains in weight produced. The only difference was the better general body condition of the animals in the flame dried group in Trial I. There was no apparent difference in body condition or physical appearance of the two groups in Trial II.

No difference was noted in the palatability of the two meals

Insofar as the results secured from this series of feeding trials are concerned, it was definitely demonstrated that there was no difference in growth-promoting properties between the two meals when fed on an equal weight basis.

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SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers
International Association of Milk Inspectors

National Dairy Experiment Station, Hillerod, Denmark
National Institute for Research in Dairying, Reading, England

New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BUTTER

The Relative Vitamin A Potency of Carotene Fed in Butter Fat and Cottonseed Oil. HENRY R. KRAYBILL AND CHARLES L. SHREWSBURY, Purdue, Univ. Agr. Exp. Sta., Lafayette, Ind. J. Nutrition *11*, p. 103, Feb., 1936.

Treatment of melted butter fat with Lloyd's reagent removes the natural yellow pigments and vitamin A without noticeable destruction or removal of the natural antioxidants.

Hydroquinone in amounts as high as 100 mg. per 100 gm. of oil does not seriously interfere with the utilization of carotene from cottonseed oil.

Carotene dissolved in butter fat decolorized with Lloyd's reagent is not utilized as well as when it is dissolved in cottonseed oil. Two to three times as much carotene are required to give equal vitamin A potency when fed in butter fat decolorized with Lloyd's reagent as when fed in cottonseed oil.

L.A.M.

The Effect of Feeding Cacao Shell to Cows on the Vitamin D Content of Butter (Milk). S. K. KON AND KATHLEEN M. HENRY, National Inst. for Res. in Dairying, Univ. of Reading. Biochem. J. *29*, p. 2051, 1935.

Cacao shell, an important by-product of chocolate manufacture, is rich in vitamin D. According to curative and preventive tests for vitamin D, cacao shell used in the feeding experiments contained an average 35 I.U. of vitamin D per gram, while the cacao shell fat, which carries about 40 per cent of the total activity of the cacao shell, contains about 300 I.U. of vitamin D per gram. The latter was about one-quarter as potent as an average cod-liver oil. A basal ration was fed four cows in winter stall feeding experiments for a pre-experimental period lasting January 8 to February 8, when for the experimental period lasting until March 17, two of the cows were each fed in addition, cacao shell increasing in amounts from one half to two pounds. An examination of the butter obtained from the milk during the experiment showed, for the control butter fat, 0.20 I.U. per gram during the pre-experimental period, and 0.21 I.U. per gram during the experimental period, whereas the "Cacao shell" butter fat showed, respectively 0.17 and 0.51 I.U. per gram. The feeding of cacao shell increased the vitamin D content of the butter fat from 2.5 to 3 times. When feeding two pounds of cacao shell per day (containing 32,000 I.U. of vitamin D) to cows on a winter ration, the vitamin D content of the butter fat is raised from a winter to a summer level.

K.G.W.

Some Practical Studies in the Cold Storage of Butter. L. C. THOMSEN, Dept. of Dairy Ind., Univ. of Wis., Madison Nat. Butter and Cheese J. *26*, 15, p. 30, Aug. 10, 1935.

Good cream and proper pasteurization are essential for cold storage butter. Recent tests show the effect of various woods for tubs and boxes on the flavor and odor of storage butter. The southern hardwoods tested are listed in order of preference:—Ash, soft maple, hackberry, sycamore, beech, yellow poplar, elm, black gum, basswood, cottonwood, red gum, and magnolia. Paraffining was of no advantage when the tubs were used for the storage of butter.

In a limited number of trials different materials used for wrapping butter were satisfactory in dark storage but in daylight (not sunlight) they protected butter from the development of oxidized flavor in the following order of effectiveness:—Coated aluminum foil; dark green, non-waterproof cellulose; cellulose with light-filtering MRT; parchment wrapped in carton; dark blue, non-waterproof cellulose; dark red non-waterproof cellulose; Kerr glass jar; waterproof cellulose; tango, non-waterproof cellulose; rubber film; pink non-waterproof cellulose; and parchment. Temperature of storage was 40° F. The butter in the best wrapper showed no oxidized flavor in one week while that in the least protective wrapper was very objectionable after three days. W.V.P.

The Effect of Certain Factors upon the Keeping Quality of Butter. E. S. GUTHRIE, B. J. SCHEIB AND C. N. STARK, Lab. of Bact., N. Y. State College of Agr., Cornell Univ., Ithaca, N. Y. JOURNAL DAIRY SCIENCE *19*, 4, p. 267, April, 1936.

The effect of milk enzymes, bacteria, acid and salt on the keeping quality of butter stored at relatively high temperatures has been studied and reported in this paper. A.C.D.

Effect of Lipolysis on the Churnability of Cream Obtained from the Milk of Cows in Advanced Lactation. V. N. KRUKOVSKY AND PAUL F. SHARP, Dept. of Dairy Industry, Cornell Univ., Ithaca, N. Y. JOURNAL DAIRY SCIENCE *19*, 4, p. 279, April, 1936.

The authors conclude that difficult churnings of cream due to cows in advanced lactation are caused directly by lipase. Prompt pasteurization of the milk or cream immediately after production prevents this difficulty in churning. A.C.D.

The Relation of the Amino Nitrogen Content to Quality of Cream and Butter. H. D. JACOBSEN, Dept. of Dairy Husb., So. Dakota State College, Brookings. JOURNAL DAIRY SCIENCE *19*, 2, p. 125, February, 1936.

The authors conclude that the amino nitrogen content of cream may be of aid in cream grading when used in conjunction with other tests.

A.C.D.

A Study of Fat Splitting and Casein Digesting Bacteria Isolated from Butter. C. N. STARK AND B. J. SCHEIB, Lab. of Bact., N. Y. State College of Agr., Cornell Univ., Ithaca, N. Y. JOURNAL DAIRY SCIENCE *19*, 3, p. 191, March, 1936.

The authors isolated and studied the characteristics of various fat splitting and casein digesting bacteria from butter. New names were suggested for five species. A.C.D.

Irradiation of Fats. I. A Standardized Method of Use of Ultra Violet Light. LESLIE H. LAMPITT, NORMAN D. SYLVESTER AND PHILIP BILHAM, Lyons Laboratories. Biochem. J. *29*, p. 1167, 1935.

The examination of the influence of light on fats and oils necessitate standard methods of evaluating the chemical changes, correlation of these results with observations of taste and smell, and a defined technique whereby figures representative of the energy of the light acting on the sample under observation can be obtained. A method is described whereby the total radiation from an electric arc applied to a sample under examination is measured by use of a photo-electric cell placed in a position other than that occupied by the sample. The method is applicable to the examination of other products wherein a knowledge of the effect of measured radiation on various properties is desired. K.G.W.

CHEESE

Bitter Flavor in Cheddar Cheese. WALTER V. PRICE, Univ. of Wis., Madison. JOURNAL DAIRY SCIENCE *19*, 3, p. 185, March, 1936.

The bitter flavor which sometimes develops in cheddar cheese was found to be due to the development of a slight excess of acid in the finished cheese as a result of too much acid development during the manufacturing process.

A.C.D.

Curd Tension of Milk and its Relationship to Firmness of Curd in Cheesemaking. G. P. SANDERS, K. J. MATHESON, AND L. A. BURKEY, Bureau of Dairy Industry, U. S. Dept. of Agr., Washington, D. C. JOURNAL DAIRY SCIENCE *19*, 6, p. 395, June, 1936.

The Hill curd test was not applicable for determining the firmness of the curd produced in milk during cheesemaking. A.C.D.

CONCENTRATED AND DRY MILKS

The Retentions of Nitrogen, Calcium, Phosphorus of Infants Fed Sweetened Condensed Milk. H. E. HARRISON. J. Ped. *8*, p. 415, April, 1936.

Metabolism tests reported by the author on infants fed on sweetened condensed milk do not support the commonly expressed belief that this product is an inadequate food for infants because of a low content of protein. On the contrary, the protein requirements of infants apparently were satisfied when they received 10 per cent of their calories in protein from sweetened condensed milk formulas, and the total intake of calories was adequate.

J.A.T.

The Antirachitic Value of Irradiated Evaporated Milk and Irradiated Whole Fluid Milk in Infants. (An in-patient study). M. RAPOPORT AND J. STOKES. J. Ped. *8*, p. 154, Feb., 1936.

Nineteen white infants under three months of age were selected for the study reported in this paper. All the infants lived in the hospital during the study. Ten of these infants were given irradiated evaporated milk and nine irradiated whole fluid milk. The two milks were practically equal in vitamin D content, the average daily intake for the evaporated milk group being 123.9 U.S.P. units daily, and for the fluid milk group 122.4 U.S.P. units daily.

During the 4½ months of the study no roentgenographic evidences of rickets appeared. Both milks were found to be equally efficacious in preventing the development of rickets. One infant showed evidence of mild rickets at the beginning of the study. Healing began in 13 weeks and was complete in 18.

J.A.T.

Irradiated Evaporated Milk in the Prevention of Rickets. T. G. H. DRAKE, F. F. TISDALL AND ALAN BROWN. J. Pediatrics *8*, p. 161, Feb., 1936.

Irradiated evaporated milk was given to 103 infants over a period of five winter months. The degree to which rickets developed in this group was compared with that observed in another group of 104 infants, half of whom received plain evaporated milk and the other half pasteurized whole milk.

In the group receiving irradiated evaporated milk the x-ray showed no cases of moderate or marked rickets. Seventeen per cent showed mild rickets but of such a slight degree as to be of no clinical importance, and all showed evidence of healing.

Of the 52 infants receiving evaporated milk with no vitamin D at the end of the period of study, 10 per cent developed x-ray evidence of moderate or marked rickets and 29 per cent showed mild rickets.

X-ray showed development of rickets to a moderate or marked degree in 28 per cent of the pasteurized milk group and mild rickets in 25 per cent of this group. J.A.T.

The Age Thickening of Sweetened Condensed Milk. V. C. Stebnitz and H. H. Sommer, Dept. of Dairy Industry, Univ. of Wis., Madison. Journal Dairy Science *19*, 2, p. 101, February, 1936.

The salts of milk were found to have an important effect upon the stability of sweetened condensed milk and sodium citrate was particularly efficient in stabilizing sweetened condensed milk which normally tended to thicken greatly during storage. A.C.D.

Nutritional Anemia in Rats Alleviated by Evaporated Milk and Iron. Herman B. Stein, Morton H. Radetsky and Robert C. Lewis, Dept. of Ped. and Biochem. of the Univ. of Colorado School of Med., Denver. Journal Dairy Science *19*, 2, p. 117, February, 1936.

The author found that the copper content of evaporated milk was sufficiently great to prevent nutritional anemia in rats when supplemented by iron alone. A.C.D.

Condensed Milk and Milk Powder. Otto Frederick Hunziker. Fifth Edition, pages 661, year 1935. Published by the author, La Grange, Illinois. Price $6.50.

Condensed Milk and Milk Powder has been carefully revised at frequent intervals since it made its appearance in 1914. The need for the present rewriting and enlarging is not only due to the rapid development in certain fields of science and in practice but must also be to a considerable extent to the desire to keep an acknowledged leading text in that recognized position by constant revision.

The author has kept the needs of the industry foremost in mind and has presented the scientific knowledge and theories with ample detail for a thorough understanding of their technical application.

In addition to presenting new material in the previous chapters, the book has also been altered by new chapters and the omission of some subjects discussed in previous editions. The chapter on packing condensed milk in vacuum has been omitted in this fifth edition. There is a new chapter on the "use of corn sugar in sweetened condensed milk," on the "manufacture of sterilized sweet cream," on "dough-and-film drying," and on the "manufacture of milk sugar."

For those not familiar with earlier editions of this book it is well to illustrate its extensive nature by the subjects covered in Part 6 on the "Manufacture of Milk Powder." There are chapters dealing not only with various phases of dry milks but also with dried buttermilk, dried whey, malted milk, and milk sugar. A.C.D.

ICE CREAM

The Effect on Quality of the Age of the Package. A. C. SCHRICKER, St. Louis Dairy Co., St. Louis, Mo. Ice Cream Field *26*, p. 18, Jan., 1935.

Some of the results of a survey made by the research committee of the Missouri Association are given in this report.

It is stated that vanilla ice cream of high quality was found to show the effects of age within four weeks from the date of manufacture, whereas chocolate ice cream required from four to six weeks while strawberry ice cream showed comparable deterioration within two weeks. Since the quality of a combination package of ice cream is no better than the worst flavor, the length of time a package of Neapolitan ice cream remains salable depends primarily on the quality of the strawberry ice cream it contains.

It is concluded that there is a direct relationship of age to quality of the ice cream, and further that the greater the percentage of old packages the greater the percentage of poor flavors. This emphasizes the value and importance of quick turnover both in the hardening room and the dealer's cabinet. W.C.

MILK

Revised Milk Designations. Lancet *230*, p. 1036; The New Grades of Milk. Edit. *230*, p. 1016, May 2, 1936.

Grading of milk was introduced in Great Britain in 1923, when Certified, Grade A (tuberculin-tested), Grade A, Pasteurized, and Grade A (pasteurized) were the designations permitted. Producers were, however, not required to conform to the standards for these grades and much milk has been sold to the public as undesignated.

On June 1, 1936, there was put into effect a New Milk Order, which provides for three grades: Tuberculin-tested, Accredited, and Pasteurized, although additional descriptions may be added by producers who are suitably qualified. Thus, there may actually be the following:

Tuberculin-tested (certified)
Tuberculin-tested (pasteurized)
Tuberculin-tested
Accredited (farm bottled)
Accredited
Pasteurized

and, of course, says the *Lancet*, undesignated milk.

Certified milk must not contain more than 30,000 bacteria per cc. Tuberculin-tested must not exceed 200,000, although after the end of the year, the bacterial plate count will be replaced with a methylene-blue reduction test. In addition, no coliform bacilli should be found in 0.01 millilitre. Tubercu-

lin-tested (pasteurized) must contain less than 30,000 organisms per millilitre. Accredited milk is raw milk from herds examined by a veterinarian every three months, and the milk must pass the same methylene-blue test as the Tuberculin-tested milk.

The *Lancet* does not consider this "accredited milk" a satisfactory product, and states editorially that the public will find safety chiefly in efficient pasteurization. J.A.T.

Effect of Tankage on the Flavor of Milk. T. M. Olson, C. C. Totman and L. C. Wallis, So. Dakota State College, Brookings. Journal Dairy Science *19*, 5, p. 313, May, 1936.

The feeding of very high proportions of tankage in the grain rations of cows has no effect upon the flavor of milk. A.C.D.

Corn Gluten Feeding and the Titratable Acidity of Milk. Elmer O. Anderson, George C. White and Robert E. Johnson, Storrs Agr. Exp. Station, Storrs, Conn. Journal Dairy Science *19*, 5, p. 317, May, 1936.

Contrary to popular opinion in certain sections of this country, the feeding of corn gluten feed in the ration of dairy cows did not affect the titratable acidity of the milk. A.C.D.

Observations on the Freezing of Milk and Cream. II. The Destruction of the Fat Emulsion in Frozen Cream. F. J. Doan and F. Bruce Baldwin, Jr. Penn. Agr. Exp. Sta., State College, Pa. Journal Dairy Science *19*, 4, p. 225, April, 1936.

This study deals with various factors involved in the freezing of cream which affect the stability of the butterfat emulsion and of the proteins. A.C.D.

Some Factors Influencing the Acidity of Freshly Drawn Cows' Milk. W. J. Caulfield and W. H. Riddell, Kan. Agr. Exp. Sta., Manhattan. Journal Dairy Science *19*, 4, p. 235, April, 1936.

The authors conclude that the acidity of fresh cows' milk is affected principally by the breed of cows, individuality of the cow and the stage of the lactation period. A.C.D.

Butterfat and Total Solids in New England Farmers Milk as Delivered to Processing Plants. Moses S. Jacobson, Lab. of the Whiting Milk Co., Botson, Mass. Journal Dairy Science *19*, 3, p. 171, March, 1936.

The fat and milk solids-not-fat was determined in more than 100,000

samples of milk produced in New England and the results were presented to establish a general relationship between these constituents. A.C.D.

Milk Production and Control. Wm. Clune, Harvey and Harry Hill. Medical Officer, and Sanitary Inspector, Public Health Department, Town Hall, Palmers Green, N. 13, England, pages 556, illustrations 180, year 1936. Published by H. K. Lewis and Co., 136 Gower St., London. Price 21s. Net.

The scope of this new book is clearly stated by the authors in the preface. The milk supply of England "affects every class of the community" and "no industry plays a more important part in the natural life of today." The authors present the general field of fluid milk production and control as it exists today in all its aspects.

The various chapters are as follows: Composition of milk, milk and disease, the cow, the cowshed, the dairy, clean milk production, the distribution of milk, designated milks, treatment of milk by heat, laboratory and other control, legislative control, future of the milk industry, and appendix.

For American readers this book has a special value in presenting a rather complete picture of the production, handling, and inspection of fluid milk from the English viewpoint. There are certain definite tendencies to stress some problems in England to which less attention is given in America and certain problems which are thought to be so important here are given minor consideration in England. A.C.D.

Fundamentals of Dairy Science. Associates of Lore A. Rogers, Bureau of Dairy Industry, United States Department of Agriculture, Washington, D. C., pages 616, Second Edition, 1935. American Chemical Society Monograph Series No. 41, Published by Reinhold Publishing Corp., 330 West Forty-Second St., New York, N. Y. Price $5.50.

A book for the "advanced student and reesarch workers in the field of dairy science" needs frequent revision. It is gratifying therefore that the authors have brought Fundamentals of Dairy Science up-to-date by a second edition within seven years of the first edition. In the preface it is stated that "knowledge of certain topics—*e.g.*, pigments, vitamins—is advancing so rapidly that, by the time this edition is published, some parts will already be in obvious need of further alteration and additions."

The organization of subject matter has not been changed, the book being divided with four parts dealing with the constituents of milk, the physical chemistry of milk and milk products, the microbiology of milk and milk products, and the nutritional value of milk and milk products grouped together with a chapter on the physiology of milk secretion. It is evident that much of the book has been completely rewritten and the net results of additional new material less that deleted is a gain of 73 pages.

The reviewer believes that students of dairy science are very appreciative of the work of the many authors who have incorporated the results of recent fundamental studies in this valuable book. A.C.D.

Variations in Serum-Magesium and in Partition of Serum-Calcium in Normal Parturition and in Milk Fever. WILLIAM GODDEN AND JOHN DUCKWORTH, Rowett Research Institute, Aberdeen. Biochem. J. *29*, p. 445, 1935.

In a study by the authors, determinations were made of serum-magnesium, serum-calcium, adsorbable and non-adsorbable calcium complexes of the serum of blood taken from nine cows prior to and after calving. Three of the cows exhibited typical symptoms of milk fever, three were abnormal cases with some signs of milk fever associated with other complications, and three were normal calvings. Generally, a normal rise in serum-magnesium was observed at calving in all three groups. In the milk fever cases a slight fall in serum-magnesium was observed followed by a definite rise persisting during the milk fever stage. A decline of serum calcium at parturition of all animals was observed, but the decline in the blood of these animals which subsequently developed milk fever was much more rapid and more extensive. A fall in the calcium complexes accounted for the major part of the fall in total serum-calcium in cases of milk fever. It appears that the symptoms of true milk fever only become acute when the values for both adsorbable and non-adsorbable calcium are simultaneously at low levels and near to their minima. With normal cases the value of the calcium complexes was lowest at about the time of calving. During recovery from milk fever-magnesium and serum-calcium rise together. K.G.W.

The Prevention of Rickets with a Cod Liver Oil Concentrate in Milk. M. G. PETERMAN AND ELY EPSTEIN. St. Vincents Infants Asylum and Dept. of Pediatrics, Marquette Univ. School of Med., Milwaukee, Wis. Am. J. Diseases of Children *50*, p. 1152, Nov., 1935.

This report concerns the use of a standard evaporated whole milk, fortified with cod liver oil concentrate during the evaporating process, in the prevention of rickets. It also includes a study of the prevention of scurvy in the same infants by the use of canned ripe pineapple juice as the sole source of vitamin C.

Each infant received from 8 to 17 ounces of the evaporated milk per day. This provided from 228 to 485 U.S.P. units of vitamin D and an additional supply of from 1142 to 2428 units of vitamin A in the added concentrate. From 1 (up to 6 months of age) to 2 ounces of pineapple juice were fed daily. A group of twenty-six infants in the susceptible age period for the development of rickets was studied from periods of from four months to thirteen and a half months (twenty infants).

Five infants had some questionable clinical signs of rickets early in the period. There were no positive roentgenographic findings and the clinical and chemical findings became normal and remained so throughout the period. All of the infants made normal gains in weight and length during the period of study. There were no signs of scurvy. W.H.R.

Physiological Bases of Nutrition. Report of a technical committee of the League of Nations. Lancet *229*, p. 1434, Dec. 21, 1935.

That deficiency in important nutrients is a common feature of the average diet and that such deficiencies are more commonly found in the protective foods rather than in the energy-yielding foods—are the conclusions of the Technical Commission of the Health Committee of the League of Nations, in a report published in Geneva.

In the first part of this report, energy requirements of different age and occupational groups are discussed, including sources of protein, fat and calories.

The second part deals with the protective foods—"first and most important of which," it is stated, "are milk and milk products, eggs and glandular tissues." Green leaf vegetables, fruits, fat fish, and meat (muscle) are also essential. The requirements of children and mothers, as well as other adults are discussed.

It is the opinion of the Commission that, "Milk should form a conspicuous element of the diet of all ages," and the report goes on to commend the tendency in some countries to encourage and provide increasing amounts of milk for pregnant and nursing women, as well as for children.

The value of skimmed or separated milk is not sufficiently recognized, the Commission believes, and it should be used far more extensively than it is, as a source of minerals, protein, calcium and vitamins B and C. Only vitamin A is reduced with the removal of the fat.

Milk is a rich source of calcium salts and phosphates, of vitamins B_2 and B_1. The calcium salts and phosphates are valuable in increasing the effect of vitamin D from whatever source. Although poor in iron itself, milk renders more effective the iron provided by other foods in the diet.

J.A.T.

Prevention of Dental Caries and the Improvement of Health by Dietary Means. F. F. Tisdall. Pa. Med. J. *39*, p. 149, Dec., 1935.

Little is known, even after the extensive studies of recent years, regarding the actual causes of dental caries. It is definitely known, however, that an adequate, well-balanced diet is an important factor in reducing, if not actually preventing tooth decay. Numerous studies of children receiving daily diets of at least 1½ pints of milk, meat, vegetables, eggs, fruits and supplementary vitamin D have shown that dental caries may be definitely

arrested. Whether such diets act by changing the resistance of the tooth itself, the composition of the saliva, or by reducing the carbohydrate in the diet is not entirely understood, but in the words of the author, "the fact remains, beyond any doubt that the administration of this type of diet does decrease tooth decay." J.A.T.

Milk As a Source of Vitamin C. Science *83*, p. 162, Feb. 14, 1936.

A note from the Kansas Agricultural Experiment Station refers to 502 tests of the vitamin C content of milk from 55 cows representing the four major dairy breeds—Holstein, Jersey, Guernsey and Ayrshire. An average value of 25.9 mg. of vitamin C per liter of milk was obtained. A range of 19 to 27 mg. of vitamin C daily has been suggested as the human minimum requirement.

These authors believe that much of the vitamin C content can be preserved in raw milk or in milk pasteurized by the flash method if proper care is exercised. J.A.T.

Study of the Committee on Clinical Investigation and Scientific Research of the American Academy of Pediatrics. 1935. J. Ped. *8*, p. 124, Jan., 1936.

Because vitamin D is essential during infancy, and only slightly less important during adolescence, pregnancy and lactation, the high cost of sources of vitamin D has been made the subject of investigation by a committee of the American Academy of Pediatrics.

In particular, the control of vitamin D sources, other than cod liver oil, by the Wisconsin Alumni Research Foundation was investigated, since this foundation owns the patents, and grants licenses both to the manufacturers of vitamin D and to food companies desiring to add this factor to their products.

Milk, bread and cereals are, according to the Foundation, the most logical foods to which vitamin D should be added. There are 135–140 licenses for irradiated fluid milk, 5 licenses for irradiated evaporated milk, and 220–225 licenses for metabolized (yeast fed) milks.

It was found by the investigators that the higher price charged for vitamin D milks over other milks, was not the result of the royalties paid to the Wisconsin Alumni Foundation but rather was necessitated by state and local regulations and other factors controlling the price of milk.

It soon became evident, according to the reporting committee, that the question was of much wider scope than was originally supposed, centering about the desirability of university patents on products relating to public health and medicine. There are two schools of thought on this matter: one group holding that any discovery or invention of benefit to the public should immediately be made available at the lowest price possible and with no re-

strictions. The other group supports the theory that certain products or activities of general value should nevertheless be strictly controlled to prevent their abuse and exploitation by ignorant or unscrupulous promoters.

In view of these and many other considerations the committee makes no recommendation in regard to the Wisconsin Alumni Research Foundation other than to urge a reduction of price on their products. J.A.T.

Food, Health and Income. A National Survey of Nutrition. Lancet *230*, p. 679, March 21, 1936. A Nutritional Survey. Brit. Med. J., p. 587, March 21, 1936.

These special articles review Sir John Orr's noteworthy study for the Rowett Research Institute and the Market Supply Committee, which has been published under the title, "Food, Health and Income. Report on a survey of Adequacy of Diet in Relation to Income."

Taking as a basis the optimum dietary standards described by Stiebeling of the U. S. Bureau of Home Economics, average diets of 1152 British families were analyzed, by dividing them into six groups according to income level. Weekly food expenditures ranged from 2 shillings per person up to 15 shillings or more.

With the exception of bread and potatoes, the consumption of essential foods, such as milk, eggs, fruit, vegetables, meat, and fish, rose as income increased. Thus, in the poorest group the average consumption of milk is equivalent to only 1.8 pints per person per week, while in the wealthiest group it is 5.5 pints.

The conclusions from this study raise important economic and political problems. To insure adequate diets to the poorer groups would involve increased consumption of milk, eggs, butter, fruits, vegetables and meat, varying from 12 to 25 per cent, and a necessarily greater food budget would be required. The newer knowledge of nutrition, indicating how there can be enormous improvement in the health and physique of the nation, has created an entirely new situation demanding "economic statesmanship."

J.A.T.

Further Observations of the Antirachitic Effect of Irradiated Fresh Milk. T. G. H. Drake, F. F. Tisdall and A. Brown. Canad. Med. Assn. J. *34*, p. 279, March, 1936.

A group of 102 infants who were under six months of age in October and November were given from ten to forty ounces daily of irradiated vitamin D milk, containing 94 international units per 20 ounces.

Clinical and x-ray studies were made over a five month period, to determine the degree of rickets, if any, in these infants. They were compared with a group of 52 infants who received ordinary pasteurized milk. All infants lived at home but their diets and general care were supervised by a competent visiting nurse.

Of the 102 infants receiving irradiated pasteurized milk at the beginning of the experimental period, 14 showed very slight rickets, 12 mild rickets and the rest no evidence of rickets. At the end of the period, in April or May, in the irradiated milk group 11 showed cured rickets and 11 healing rickets, while of the non-irradiated milk group, 17 showed active rickets and seven healing rickets. J.A.B.

Further Observations on the Comparative Antirachitic Value of Crystalline Vitamin D Administered in Milk, Corn Oil or in Propylene Glycol. J. M. Lewis. J. Pediatrics *8*, p. 308, March, 1936.

It has been shown in previous studies that when milk is used as a carrier for vitamin D, a lower unitage will bring about more satisfactory healing of rickets than when the vitamin D is given in some other medium, such as corn oil.

In this paper the prophylactic value of 145–290, and 1450 U.S.P. units of crystalline vitamin D given in each of three mediums is reported. The three mediums were milk, corn oil and propylene glycol. The total number of infants studied was 355.

At the end of the study the two groups receiving vitamin D in milk showed a lower incidence of rickets than did any of the other groups. No infant in the milk groups developed moderate or marked rickets, while four infants in the other groups showed evidence of more advanced rickets.

The author discusses possible reasons why milk appears to be a superior carrier for vitamin D, and advances two suggestions: one, that the combination of the calcium and phosphorus of the milk with the vitamin D may be advantageous; and two, that when milk is used as a carrier the infant gets vitamin D in small amounts with every feeding, whereas when corn oil or propylene glycol is used as a carrier, the vitamin D is given in a single dose only once a day. This theory is being investigated further. J.A.T.

The Component Fatty Acids of Goat Milk. R. W. Riemenschneider and N. R. Ellis. Bur. of Animal Ind., U. S. Dept. of Agr., Washington, D. C. J. Biol. Chem. *113*, 1, p. 219, Feb., 1936.

A study was made of the nature and composition of a composite sample of 3.5 kilos of filtered butterfat prepared from the milk from a goat herd maintained on a regulated dietary régime. The butterfat was converted to methyl esters, and fractionated into 63 fractions for examination and determination of the component fatty acids.

In addition to the saturated acids of an even number of C atoms from butyric to stearic, and the unsaturated oleic acid, the presence of a number of other fatty acids was established. These included the unsaturated acids decenoic, tetradecenoic, hexadecenoic, and probably arachidonic, and the higher molecular weight saturated acids lignoceric and cerotic. Traces of

an octobromide of an unknown acid were found, probably of a C_{22} acid or an impure isomer of arachidonic acid. Estimations on the content of the component fatty acids present show general agreement with previously published analysis of the composition of goat fat, except for the absence of linoleic acid, and the presence of small percentages of decenoic acids noted above. K.G.W.

Investigations on the Vitamin B_2 Complex. II. The Distribution of Lactoflavin and of the "Pellagra-Preventing Factor" Vitamin B_6 in Natural Products of Animal Origin. PAUL GYÖRGY, Physiological and Nutritional Laboratories, Cambridge. Biochem. J. *29*, p. 760, 1935.

The author in this paper has determined the relative amounts of the two substances in a number of products. Concerning milk, the observation is reported that cow's milk (summer) contains about the same amount of lactoflavin as of vitamin B_6. K.G.W.

Investigations on the Vitamin B_2 Complex. III. The Inactivation of Lactoflavin and Vitamin B_6 by Visible Light. PAUL GYÖRGY, Physiological and Nutritional Laboratories, Cambridge. Biochem. J. *29*, p. 767, 1935.

Both lactoflavin and the pellagra-preventing substance (vitamin B_6) obtained from a concentrate from cow's liver, may be destroyed by the visible light obtained from a 500 k.w. electric bulb. K.G.W.

A Discrepancy Between Biological Assays and other Methods of Determining Vitamin A. RONALD S. MORGAN, Lever Bros. Ltd., Port Sunlight, England, and Joseph R. Edisbury and Richard A. Morton, Univ. of Liverpool. Biochem. J. *29*, p. 1645, 1935.

A comparison is made of biological assays for vitamin A, Lovibond Blue values, and spectroscopic estimates of the percentage of vitamin A present for a series of 22 oils and concentrates covering a range of potency of 530 to 1,290,000 I.U. per gram. Extensive statistical analysis has been made of the data to ascertain the variation in results by the different methods. K.G.W.

The Comparative Antirachitic Efficiency of Vitamin D in Irradiated Milk, Metabolized (yeast) Milk and Cod Liver Oil. R. M. BETHKE, W. E. KRAUSS, P. R. RECORD AND O. H. M. WILDER. Ohio Agr. Exp. Sta., Wooster, Ohio. J. Nutrition *11*, p. 21, Jan., 1936.

It required more than ten times the rat equivalent amount of vitamin D in metabolized (yeast) milk than in irradiated milk to produce the same antirachitic effect in chicks.

Equivalent rat units of vitamin D from cod liver oil and irradiated milk were equally efficient, antirachitically, for the chicken.

The data indicate that the vitamin D in milk resulting from feeding irradiated yeast to the cow (metabolized milk) is in the same biological form as that fed to the animal.

Since rat equivalent amounts of vitamin D in metabolized (yeast) milk and irradiated milk were found equally efficacious for the rachitic infant and not for the chick, it is concluded that the infant and chick vary greatly in their response to the forms of vitamin D in these two types of milk.

L.A.M.

The Relation of Sodium to Chlorine in the Milk of Shorthorn and Guernsey Cows. TUDOR S. G. JONES AND WILLIAM L. DAVIES, National Institute for Research in Dairying, Shinfield. Biochem. J. *29*, p. 978, 1935.

The milk of each of twenty cows of the shorthorn and Guernsey breeds was examined to correlate the sodium and chloride contents of their milk and their period of lactation. The values for sodium and chloride ranged from 39.2 and 139.2 and 70 to 193 mg. per 100 ml. respectively, with mean values of 76.8 and 113.4 mg. per 100 ml. respectively. The relation between chloride and sodium in milk of the two breeds examined is given by the equation $Cl - 1.24\ Na + 18.09$, wherein Cl and Na are expressed in mg. per 100 ml. Inasmuch as the chloride content of milk is known to increase with advance in the period of lactation, an increase in the sodium content is to be expected. The limited number of examinations did not permit statistical correlation of the Na values with advance in lactation. K.G.W.

The Effect of Halogen Salts on the Clotting of Milk by Trypsin. WINIFRED M. CLIFFORD, Physiology Dept., King's College of Household and Social Science, Campden Hill Road, W.8. Biochem. J. *29*, p. 1059, 1935.

When trypsin is added to milk (1 ml. of 2 per cent pancreas substance to 10 ml. of milk and 2 ml. of water) the appearance of the mixture usually remains unaltered. The addition of Ca salts to the mixture is known to affect the action to the extent that a typical firm coagulum can be obtained. An investigation of the influence of halogens (Fl, Cl, Br, I) in the form of salts of Li, Na, K, NH_4, Ca, Mg and Ba indicate that the acceleration of tryptic activity in clotting of milk when $CaCl_2$ is added is as much a function of the halogen as of the Ca radical. The halogen salts of the alkaline earths were found more effective in aiding coagulation than those of Li, Na, K and NH_4; the coagulating action was least with fluorides. The efficacy of the alkaline earths follows the order $Ca > Ba > Mg$. K.G.W.

Relative Value of Raw and Heated Milk in Nutrition. E. C. V. MATTICK AND J. GOLDING, National Inst. for Res. in Dairying, Reading, Eng. Lancet *230*, p. 1132, May 16, 1936.

The authors report on a continuation of feeding experiments with rats begun several years ago and first described in 1931 (Lancet, March 21, 1931). Rats from the same litters were placed at weaning on diets of biscuit made from flour and water, plus (a) raw milk, or (b) "freshly" sterilized milk, or (c) "kept" sterilized milk. The "freshly" and "kept" sterilized milks are not defined in this article, but in the 1931 paper, the freshly sterilized milk was stated to be raw milk which had been heated to 210–212° F. for half an hour and fed the same day, whereas the "kept" sterilized milk had been heated in the same manner, but was fed 24 hours later.

Rats of the raw milk group were weaned on the first matings through the seventh generation, but no second generation was weaned on the "kept" sterilized milk, and no third generation on the freshly sterilized milk. After the third generation of the raw milk group, no significant differences in weight were found, and there were no dental lesions in the seventh generation animals.

The practical significance, if any, of this limited experiment is not stated. J.A.T.

The Metabolism of Galactose. II. The Synthesis of Lactose by Slices of Active Mammary Gland *in Vitro*. GORDON ALLISON GRANT, Dept. of Biochem., Lister Inst., London. Biochem. J. *29*, p. 1905, 1935.

One of the interesting transformations in carbohydrate metabolism is that occurring in the active mammary gland during the synthesis of the galactose-containing disaccharide, lactose. While many studies have been made concerning the nature of the transformation, as well as the source from which lactose is derived, the available physiological evidence suggests that the freely lactating gland is apparently capable of withdrawing glucose from the blood, and converting it into the milk sugar, lactose. In this investigation lactose synthesis has been demonstrated and quantitatively investigated with tissue slices of the active mammary gland maintained *in vitro* under conditions as nearly as possible physiological, using as substrates the hexoses, glucose, fructose, mammose and galactose. Of the hexoses used, glucose is readily converted into lactose, while there is but little evidence of synthesis from fructose, mammose and galactose. K.G.W.

Lactoflavin, a Possible Contaminant of Vitamin-Free Diets. G. C. SUPPLEE, G. E. FLANIGAN, ZAIDA M. HANFORD, AND S. ANSBACHER, Dry Milk Co., Bainbridge, N. Y. J. Biol. Chem. *113*, p. 787, 1936.

Casein is the basic protein in many rations used in determination of

vitamins associated with biological materials and foodstuffs. While essentially it is desired that the casein be purified and free of associated factors, it is possible that certain factors with growth promoting properties may be present. Lactoflavin has recently been shown to possess such properties, and has been found associated with certain types of casein.

The investigators showed that lactoflavin as identified by its characteristics of fluoresence in "black light," is a contaminant of crude or commercial caseins, and even certain "purified vitamin free caseins." The presence of lactoflavin in commercial caseins after extraction for extended periods with acetic acid and alcohol may be readily revealed by examination of the fluoresence in "black light." If the casein is prepared by a process involving a six step elution treatment with weak sodium chloride solution at the isoelectric point of the casein, a product entirely free of lactoflavin is obtained. The variation in the growth promoting properties of various caseins used in rations fed white rats in the experiments reported was attributed to the relative contents of lactoflavin.

The Influence of Storage, Pasteurization, and Contamination with Metals on the Stability of Vitamin C in Milk. C. H. WHITNAH, W. H. RIDDELL, AND W. J. CAULFIELD, Kan. Agr. Exp. Station, Manhattan. JOURNAL OF DAIRY SCIENCE *19*, 6, p. 373, June, 1936.

As a result of the studies on the stability of vitamin C in milk, the authors conclude that either raw or high temperature pasteurized milk if reasonably fresh and uncontaminated with copper may be an important source of vitamin C in human nutrition. A.C.D.

Effects of Time and Temperature of Holding Milk Heat-Treated at Various Temperatures Upon its Subsequent Coagulation by Rennet. MILTON E. POWELL, Div. of Agr. Biochem., Univ. of Minn., St. Paul. JOURNAL OF DAIRY SCIENCE *19*, 5, p. 305, May, 1936.

This study dealt primarily with the slight change which occurs in the rennet coagulation of pasteurized milk during aging subsequent to pasteurization. A.C.D.

The Effect of the Adsorption "Membrane" Around the Fat Globules on the Curd Tension of Cow's Milk. L. S. PALMER AND N. P. TARASSUK, Div. of Agr. Biochem., Univ. of Minn., St. Paul. JOURNAL OF DAIRY SCIENCE *19*, 5, p. 323, May, 1936.

The authors conclude that the "membrane" adsorbed on the fat globules in milk has a definite effect in lowering curd tension. A.C.D.

Normal Variations in the Curd Tension of Milk. W. H. RIDDELL, W. J. CAULFIELD AND C. H. WHITNAH, Kan. Agr. Exp. Sta., Manhattan. JOURNAL OF DAIRY SCIENCE *19*, 3, p. 157, March, 1936.

The breed of cows, the individuality of the cow, and the stage of the

lactation period were of major importance in determining the curd tension of the milk. A.C.D.

The Fluorometric Estimation of Lactoflavin. G. C. SUPPLEE, S. ANSBACHER, G. E. FLANIGAN, AND Z. M. HANFORD, Res. Lab. of the Dry Milk Co., Inc., Bainbridge, N. Y. JOURNAL OF DAIRY SCIENCE *19*, 3, p. 215, March, 1936.

A method was presented for estimating lactoflavin in milk by its fluorescent properties in "black light." A.C.D.

Soybean Flour as a Substitute for Cow's Milk in Feeding Dairy Calves. LAVAN SHOPTAW, Panhandle Agr. Exp. Sta., Goodwell, Oklahoma. JOURNAL OF DAIRY SCIENCE *19*, 2, p. 95, February, 1936.

Contrary to some statements in the literature the author failed to secure as good results with soybean flour as was secured from cow's milk in raising dairy calves. A.C.D.

Is Bovine Mastitis a Public Health Problem? PAUL B. BROOKS, N. Y. St. J. Med. p. 584, April 1, 1936.

The control of bovine mastitis is more of an economic than it is a public health problem, in the opinion of the author. Many milk-borne epidemics are traceable to cows with mastitis, but "the cases of mastitis known to be dangerous to human health are those in which the infecting organisms are transmitted to the cows' udders from infected persons," such as milkers. Eradication of mastitis would not prevent such infections, and milk-borne epidemics could occur before even a careful dairyman could recognize the condition as present in his herd. "Pasteurization, therefore, is the only dependable safeguard" to prevent such outbreaks. J.A.T.

Formaldehyde as a Selective Bacteriostatic Agent. W. J. WILSON. J. Path. Bact. *40*, p. 199, 1935.

In an attempt to find a selective medium for the isolation, cultivation and differentiation of *B. coli* and *B. lactis aerogenes*, 2 cc. of a 40 per cent solution of hexamine was added to lactose peptone water. It was believed that the bacteriostatic action of the hexamine was due to the liberation of formaldehyde since formaldehyde had a similar action. With heavy implantations the strength of formaldehyde used was 1:20,000 and 1:50,000, whereas with light implantations, as in water, one-half these concentrations were used. Readings were taken at the end of 24 hours. Experiments showed that in lactose-peptone-water medium containing 5 cc. of a 1:40 watery solution of hydroquinine and 5 cc. of 1:1000 formalin the growth of fecal *B. coli* was profuse and that of *B. lactis aerogenes* suppressed. The reagents were added just before the medium was used. The author hopes

to be able to use this method in determining fecal *B. coli* contamination in milk, water, foods, etc. F.W.F.

New Culture Media Based on Sodium Desoxycholate for the Isolation of Intestinal Pathogens and for the Enumeration of Colon Bacilli in Milk and Water. EINAR LEIFSON, J. Path. Bact. *40*, p. 581, 1935.

The source and general chemical nature of desoxychloic acid and its salts are discussed. This acid and its salts are compared with some of the other acids found in bile. The effect of desoxychloic acid on bacteria together with the effective pH ranges for different bacteria are given. These data are represented graphically in the form of a chart. The effect of various substances such as the salts of the straight-chain fatty acids, the higher aliphatic acids, dyes, iron salts, and raw milk on desoxycholate infusion agar are discussed.

Directions are given for making up desoxycholate agar and the influence of the different ingredients upon the medium. The medium best suited for enumerating the colon bacilli in milk and milk products is discussed in detail. According to the author, colon bacilli grow rapidly in the desoxycholate agar and appear as large, red colonies in 15 to 20 hours, whereas other colonies are barely visible in this time. *Proteus* colonies are slightly pinkish and are surrounded by a clear area due to the hydrolysis of the casein in the milk. The *Proteus* colonies are so characteristic that this medium may also be used to count them in the milk. By decreasing the concentration of lactose, the deep colonies of *Aerobacter* generally become quite colorless in 24 hours. Some difficulty may be experienced in differentiating between the surface growth of the colon bacilli and other bacteria.

The author describes many other uses which may be made of the desoxycholate agar when combined with sodium and ferric ammonium citrates and also when P_6 peptone is used. Fecal streptococci and enterococci and many other types of streptococci may also be isolated by changing the pH. Flagellated bacilli become non-flagellated as long as they are kept in contact with desoxycholate but regain their flagella when transferred to other media. F.W.F.

Evaluation of Certain Media for the Detection of Colon Organisms in Milk. C. N. STARK AND L. R. CURTIS, Lab. of Bact., Cornell Univ., Ithaca, N. Y. Am. J. Pub. Health *26*, 4, p. 354, April, 1936.

The authors found that crystal violet, Dominick-Lauter and gentian violet broths were not satisfactory for use in the detection of colon organisms in 1 cc. quantities of milk, due to growth of a considerable number of false test organisms. Brilliant green bile broth had a better selective action which was partially destroyed by the protein material in 1 cc. amounts of milk used as inoculum.

Formate ricinoleate broth can be successfully used for the detection of colon organisms in milk because: (1) it inhibits the growth of false test-organisms; (2) it permits growth and gas production by small numbers of *Escherichia-Aerobacter* organisms; (3) it results in increased bacterial growth and gas production by maintaining a favorable environment for growth; and (4) it is little affected by the additional protein material which must be added when inoculating media with 1 cc. amounts of milk. Of the bacteria tested which were able to grow in formate ricinoleate broth, only the *Escherichia-Aerobacter* and *Salmonella* groups were able to produce gas from sodium formate. The authors believe that ability of the *Salmonella* group to produce gas from sodium formate is an advantage rather than a disadvantage, since the presence of these bacteria in milk should not be tolerated. M.W.Y.

Optimum Temperature of Incubation for Standard Methods of Milk Analysis as Influenced by the Medium. M. W. YALE, AND CARL S. PEDERSON. N. Y. Agr. Exp. Sta., Geneva, N. Y. Am. J. Pub. Health *26*, 4, p. 344, April, 1936.

The authors have previously recommended an incubation temperature of 32° C. for 48 hours rather than 37° C. for standard beef-extract agar plates prepared from samples of dairy products since they found 32° C. to be a fairer and truer measure of quality than 37° C. incubation.

The possibility that the present standard agar may be replaced by a medium better suited for the growth of bacteria found in milk led the authors to study the optimum 48-hour incubation temperature for the tryptone-glucose-skimmilk agar proposed by Bowers and Hucker.

The optimum temperature in the case of raw milk was slightly below 30° C. and in the case of pasteurized milk slightly above 31° C. The conclusion is drawn that an incubation temperature of 32° C. or slightly lower is fully as desirable with tryptone agar as with the present standard agar because, (1) it yields at least 95 per cent on the average of the maximum 2-day count in comparison to only about 50 per cent in the case of 37° C. incubation, (2) less errors are caused by temperature variations in 32° C. than in 37° C. incubators; and (3) the percentage of the maximum 48-hour count does not vary greatly between samples in the case of 32° C. incubation whereas it varies greatly in the case of 37° C. incubation. M.W.Y.

Further Studies of the Composition of Media fo the Bacteriological Analysis of Milk. C. S. BOWERS, Conn. State Dept. Health Lab., Hartford, Conn., and G. J. HUCKER, N. Y. Agr. Exp. Sta., Geneva, N. Y. Am. J. Pub. Health *26*, 4, p. 350, April, 1936.

Earlier work by these authors (Tech. Bul. 228, N. Y. Exp. Sta.) suggested that an agar containing tryptone (a commercial form of digested

casein), glucose and skimmilk should be substituted for the peptone-beef extract agar now accepted as the standard agar for milk work, since on the average, this medium increased the count above that on standard agar by 33 per cent when raw milk samples were studied and 147 per cent when pasteurized milk was used. The data obtained in the present study substantiates the above conclusion.

Increases in count were irregular due to the fact that samples contained different proportions of the types of bacteria which grew poorly or not at all on standard beef extract agar.

The additon of either skimmilk or glucose to standard agar materially increased its efficiency although such a modification did not result in standard agar becoming as efficient a plating medium as tryptone-glucose agar or tryptone-glucose skimmilk agar. Tryptone-glucose agar was not quite as efficient as the same agar to which skimmilk was added. Tryptone was approximately 37 per cent more efficient than neopeptone (a second commercial form of digested casein) from the standpoint of number of colonies developing on plates made from pasteurized milk. M.W.Y.

The Detection of Mastitis in Dairy Herds. D. H. JACOBSEN, AND T. M. OLSON, Bul. 290, 1935, So. Dak. State College, Brookings.

The economic aspects, causes and control of mastitis are discussed. The college herd consisting of 54 cows was checked for a period of 16 months. Of the 54 cows, 14 produced milk which gave positive tests and was physically abnormal, containing clots or sediment or was serous or thick in consistency. Twenty-seven were positive to the Brom thymol blue and catalase tests and had high leucocyte counts. Twenty-four of the 27 showed either long-chain streptococci or staphylococci in the milk at some time during the trials. Thirteen cows which were termed suspicious to positive but never showed milk of abnormal consistency during the experiment gave positive laboratory tests as follows: Of 450 "quarter" samples tested 174 or 32.2 per cent were positive to the Brom thymol blue test. One hundred ninety-two or 35.6 per cent showed more than 500,000 leucocytes per cc. Two hundred fifty-two or 46.7 per cent produced more than 1.5 cc. gas by the catalase test. Eighty-eight or 16.3 per cent showed long chain streptococci incubated samples.

Conclusions:

1. Laboratory tests were a valuable aid in detecting cases of chronic mastitis which would have passed unnoticed by the milkers.

2. Because of the mild and recurrent nature of the infection the tests must be used at frequent intervals to detect and eliminate dangerous sources of infection.

3. Infection is frequently confined to one or more quarters of an udder. Therefore the detection of milk from such quarters is necessary in controlling the spread of infection.

4. Brom thymol blue tests, catalase test, and cell counts all seem to be satisfactory tests for the detection of mastitis. The Brom thymol blue test is the simplest in operation and is recommended for dairy herd control.

5. Microscopic examination of stained smears is not a good routine method of detecting "mastitis" milk when used alone because a relatively low percentage of the samples from abnormal udders contain long-chain streptococci.

6. Positive Brom thymol blue, catalase and high cell count do not definitely prove mastitis infection but the tests are an indication of abnormal milk which is at least presumptive evidence of an abnormal udder condition.

C.C.T.

Testing of Abortus Vaccines and their Protective Action against the Disease in Cattle. H. ZELLER AND W. STOCKMAYER, Infektion. Parasitäre Krankheiten Hygiene Haustiere *48*, 1/2, p. 77, 1936.

Several vaccines were studied but these investigators were not able to establish the value of them during the first pregnancy period. They were perhaps of slight value in reducing sterility during the second period. There also appeared to be little difference between the number of abortus bacilli in the organs of treated and control animals after slaughter. At the close of the second year of the experiment 32 cows and one bull were slaughtered. Of the cows, 30 gave positive reactions by the agglutination test and only 19 were positive by the complement-fixation test. The three animals negative to all tests were proved not to be carriers of the abortion bacillus on post-mortem examination. About half of the 40 animals studied did not yield positive cultures from their internal organs. Of the 22 which were positive, 18 were found to be infected in the udder and 15 in the udder-lymphatics. Five yielded the abortion bacillus from the intestinal-lymphatics. Three of these animals had given normal births. This is thought to indicate that the abortion bacillus may disappear from the uterus but not from the udder. The organisms were not found in the liver or bone marrow in any of the infected animals. They were found once in the spleen, and mesenteric lymphnodes, in the retropharyngeal lymph nodes in 2 animals.

From the fact that these organisms establish themselves in the udder and are able to persist for such long periods, and are of importance from a public health standpoint, the writers are opposed to the use of live-germ vaccines of an attenuated type for the vaccination of cows. The control of the disease must depend on the application of serological tests and hygienic measures for its control.

L.D.B.

Investigation of the Use of Entozon in Mastitis. Infektion. Parasitäre Krankheiten Hygiene Haustiere *48*, 1/2, p. 95, 1936.

These tests were conducted on several herds of dairy cows which were found to be infected in the udder by streptococci. The usual methods were

used to diagnose the condition. When a diagnosis was certain the quarter was freed of milk and injected with about 100 cc. of a 1 to 1250 warmed solution of Entozon. This fluid is then removed and again treated in the same manner. Care is observed not to inject too much fluid. This is followed by slight massage. The infusion remains in a dry animal for 20 to 24 hours, in lactating animals 3 to 5 minutes. The use of this method has proved to be quite favorable as a curative agent in quarters where there is a minimum of pathological change. Many animals are treated 3 to 5 times per week in this manner.

The author emphasizes the value of sanitation and quarantine in the control of this disease. He advocates giving careful instructions to the owners and milkers of infected herds. L.D.B.

Some Effects of the Proposed New Bacteriological Techniques. Joseph F. Phelan, Lab. of H. P. Hood and Sons, Inc., Boston, Mass. Journal of Dairy Science *19*, 6, p. 385, June, 1936.

Incubation of tryptone glucose skimmilk agar at 32° C. produces higher bacterial counts and Standard Methods of Milk Analyses should not be altered to affect the bacterial count without careful consideration to present bacteriological standards. A.C.D.

Detecting Recontamination of Pasteurized Milk by Bacteriological Methods. W. H. Chilson, M. W. Yale, and R. Eglinton, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Journal of Dairy Science *19*, 5, p. 337, May, 1936.

Since properly pasteurized milk free from recontamination contains less than 1 colon organism per cc. it is evident that the test for the colon bacteria may be used to supplement the results secured by the standard agar plate count in determining recontamination of pasteurized milk. A.C.D.

Rennet Test for the Detection of Mastitis. F. B. Hadley, Dept. of Vet. Science, Univ. of Wis., Madison. Journal of Dairy Science *19*, 3, p. 165, March, 1936.

The author concludes that the time required for milk to coagulate with rennet is a valuable test to aid in detecting mastitis. A.C.D.

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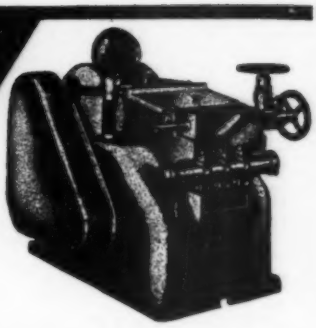

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